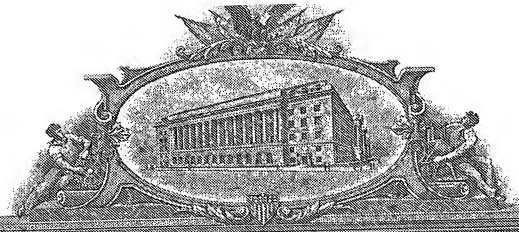

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THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: ***60/500,708***
FILING DATE: ***September 05, 2003***
RELATED PCT APPLICATION NUMBER: ***PCT/US04/29047***

Certified by

W. Dudas

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Express Mail No.: EV 124826252 US
Attorney Docket No.: 2003080-0134
(SK-1062-PRO3)
Date Filed: September 5, 2003

CERTIFICATE OF EXPRESS MAILING

"Express Mail" mailing label number EV 124826252 US
Date of Deposit: September 5, 2003

I hereby certify that this correspondence is being deposited with the United States Postal Service as ***"Express Mail Post Office to Address"*** service under 37 CFR 1.10 on the date indicated above and is addressed to: Commissioner for Patents, Mail Stop Provisional Patent Application, P.O. Box 1450, Alexandria, VA 22313-1450

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Sandy Sliger
Typed Name

PROVISIONAL APPLICATION TRANSMITTAL

(REQUEST FOR FILING A PROVISIONAL APPLICATION FOR PATENT UNDER 37 CFR § 1.53(C))

Dear Sir:

Please find enclosed a provisional patent application and papers as follows for:

Inventor(s):

Given Name (first and middle)	Family Name or Surname	Residence (City and State or Foreign Country)
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Justin	Miller	New York, NY
J. David	Warren	New York, NY

Title of the Invention: **GP120 SPECIFIC ANTIGENS, CONJUGATES THEREOF, METHODS FOR THEIR PREPARATION AND USES THEREOF**

A) ENCLOSED APPLICATION PARTS:

1) X	**Specification**	PAGES: 129
2) X	**Claims**	PAGES: 36
3) X	**Abstract**	PAGES: 1
4) X	**Drawing(s)**	SHEETS: 1
5) X	**Appendix A**	PAGES: 30
6) X	**Appendix B**	PAGES: 29
		TOTAL: 226

B) OTHER ACCOMPANYING APPLICATION PARTS:

3) X Return Receipt Postcard (MPEP § 503) (specifically itemized)

4) __ Application Data Sheet. See 37 CFR § 1.76

5) __ **OTHER:** (if applicable, specified below)

C) GOVERNMENT SUPPORT:

THE INVENTION WAS MADE BY AN AGENCY OF THE UNITED STATES GOVERNMENT OR UNDER A CONTRACT WITH AN AGENCY OF THE UNITED STATES GOVERNMENT:

____ NO

__X__ YES, THE NAME OF THE U.S. GOVERNMENT AGENCY AND THE GOVERNMENT CONTRACT NUMBER ARE : **BC020513 and BC022120 - US ARMY (DOD) BREAST CANCER RESEARCH FOUNDATION**

D) METHOD OF PAYMENT OF FILING FEES:

X Applicant claims small entity status. See 37 CFR §1.27.

___ Statement Verifying Small Entity Status (optional)

X A check or money order is enclosed to cover the filing fees

X The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-1721.

FILING FEE AMOUNT ($): **80.00**

E) CORRESPONDENCE ADDRESS:

X Customer Bar Code Label: 
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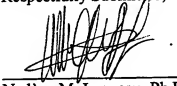

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GP120 SPECIFIC ANTIGENS, CONJUGATES THEREOF, METHODS FOR THEIR PREPARATION AND USES THEREOF

RELATED APPLICATIONS

[0001] This application is related to provisional application No.: 60/430,822, filed December 3, 2002, entitled "Novel Glycoconjugates, Glycopeptides, Glycoproteins, Intermediates Thereto, Methods for Preparing These and Uses Thereof"; and provisional application No.: 60/_____, filed September 4, 2003, entitled "Prostate Specific Antigens, Conjugates Thereof, Methods for their Preparation and Uses Thereof"; each of which is hereby incorporated by reference in its entirety.

GOVERNMENT SUPPORT

[0002] The invention was supported in part by Grant Nos.: BC020513 and BC022120 from the US Army (DOD) Breast Cancer Research Foundation. The U.S. government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Despite enormous scientific effort, the development of a vaccine against HIV has proven to be a largely elusive goal. There are several major factors complicating the creation of such vaccine.

[0004] One problem stems from a very low immunogenicity of the viral surface. Pairs of the envelope spike proteins (gp120 and gp41) form a trimer, inside of which much of the potentially antigenic surface of the unprocessed precursor protein (gp160) is buried. Moreover, the "outer" face of gp120 is extensively glycosylated (and therefore unavailable for peptide - recognizing antibodies), further complicating the problem.

[0005] Secondly, the mature envelope oligomer is itself a very weak antigen. Many explanations have been proposed to explain the unusually low antigenicity of the viral envelope spikes. The "glycan shield" concept implies that steric hindrance created by *N*-linked carbohydrates of gp120 prevents the immune system form generating antibodies with a broadly neutralizing action. Another hypothesis states that binding of neutralizing antibodies to the CD4 site of gp120 leads to conformational changes and is entropically disfavored, thereby allowing

for HIV neutralization escape. It has also been suggested that a very strong initial immune response to gp160, which does not lead to broadly neutralizing antibody production (vide supra) suppresses response to the mature oligomer, which is expressed in much lower concentrations.

[0006] In addition, extremely high degree and rate of viral variation provide a powerful mechanism for HIV to escape immune defense.

[0007] Accordingly, commonly utilized vaccine formulations have been unable to elicit potent and broadly neutralizing antibody response. Administration of the whole virus in attenuated or inactivated form presents safety issues as well as the problem of low antigenicity. Immunization with a part of HIV DNA in a carrier is more promising, however it requires a very careful choice of the carrier virus. Also, low envelope antigenicity still remains a serious obstacle to the success of this method. A solution may lie in the use of artificial HIV antigens based on the epitopes of known broadly neutralizing antibodies. A highly focused immune response may be developed with this approach, potentially circumventing the problem of low antigenicity. The biggest challenge in this case is the design and synthesis of the antigens.

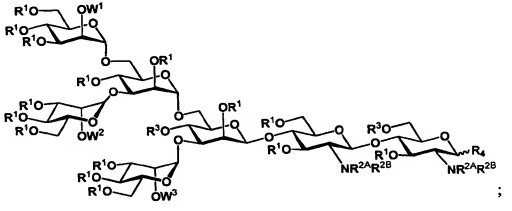
[0008] Gp120 surface carbohydrates can be seen as an attractive target for such design. There are a number of molecules that can efficiently bind to HIV envelope glycans. Among them, the dendritic cell receptor DC-SIGN has been demonstrated to recognise the internal tri-mannose segment of the N-linked oligosaccharides. A bacterial protein cyanovirin-N efficiently binds high-mannose type gp120 carbohydrates. Also, one of the most potent broadly neutralizing antibodies known to date, the 2g12, has been shown to have a carbohydrate epitope. Administering *synthetic antigens containing one or more glycans on a part of gp120 peptide backbone or appropriately chosen linker system and further conjugated to an antigenic carrier* could elicit strong immune response ultimately aimed at the real viral envelope. Some of the *N*-linked carbohydrates of gp120 appear to be conserved in most of HIV primary isolates. Since the glycans recognized by these molecules are located on the outer, "silent" face of the oligomer, they are easily accessible for antibody binding. Entropically disfavored interaction does not present a problem as well since the epitope does not overlap with the CD4-binding site. Finally, an extensive glycosylation of the envelope is an advantage, rather than a problem for such antigen design.

[0009] Accordingly, there remains a need for novel synthetic methods leading to the preparation of GP120 glycans and conjugates thereof, and their evaluation in immunologic and therapeutic studies.

SUMMARY OF THE INVENTION

[0010] In recognition of the need to provide access to synthetically unavailable GP120 glycans and glycopeptides, the present invention, in one aspect, provides novel GP120 glycans and N-linked peptide conjugates thereof, and methods for the synthesis and use thereof.

[0011] In one aspect, the present invention provides compounds having formula **(I)**:

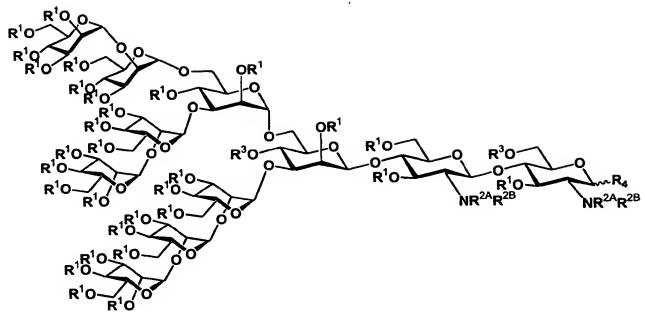


;

(I)

wherein R^1, R^{2A}, R^{2B}, R^3, R^4, W^1, W^2 and W^3 are as defined herein.

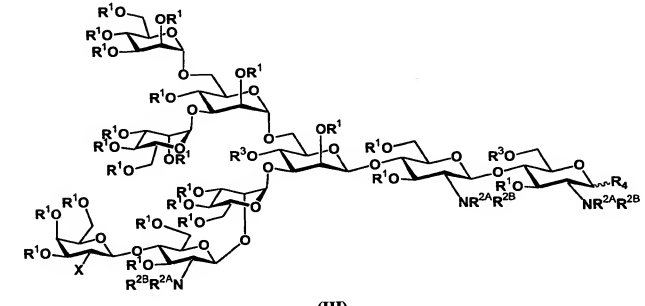
[0012] In certain embodiments, the invention provides a compound of formula **(II)** having the structure:



(II)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined herein.

[0013] Incertain embodiments, the invention provides a compound of formula (III) having the structure:



(III)

wherein X, R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined herein.

[0014] In another aspect, the invention provides compositions of any of the compounds disclosed herein. In certain embodiments, a pharmaceutical composition is provided comprising

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an effective amount of a compound of formula **(I)**, **(II)** and/or **(III)** in admixture with a pharmaceutically suitable diluent or carrier. In certain other embodiments, a composition is provided for eliciting an immune response in a subject comprising an effective amount of a compound of formula **(I)**, **(II)** and/or **(III)** in admixture with a suitable immunogenic stimulant.

[0015] In another aspect, the invention provides methods for the use thereof in the treatment of HIV, methods for the prevention of HIV, and methods for inducing antibodies in a subject, comprising administering to a subject in need thereof, an effective amount of any of the inventive compounds as disclosed herein, either in conjugated form or unconjugated and in combination with a suitable immunogenic carrier.

[0016] As detailed herein, in another aspect of the present invention, any of the inventive compounds may be conjugated to generate a glycoconjugate, and may be administered alone, with an immunogenic carrier (for example, a carrier protein, peptide or lipid), or with an immunological adjuvant or any combination thereof for the treatment of HIV infection and/or for preventing HIV infection, or may be administered alone or with an immunological adjuvant to induce antibodies in a subject.

DEFINITIONS

[0017] Certain compounds of the present invention, and definitions of specific functional groups are also described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference.

[0018] It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the

same or different at every position unless otherwise indicated. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment and/or prevention of HIV, or in the inducement of antibodies, as described herein. The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein.

[0019] The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched) or branched aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl moieties. Thus, as used herein, the term "alkyl" includes straight and branched alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl" and the like. Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl" and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

[0020] In certain embodiments, the alkyl, alkenyl and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-

propyl, isopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, n-hexyl, sec-hexyl, moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-l-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

[0021] The term "alicyclic", as used herein, refers to compounds which combine the properties of aliphatic and cyclic compounds and include but are not limited to cyclic, or polycyclic aliphatic hydrocarbons and bridged cycloalkyl compounds, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "alicyclic" is intended herein to include, but is not limited to, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties, which are optionally substituted with one or more functional groups. Illustrative alicyclic groups thus include, but are not limited to, for example, cyclopropyl, $-CH_2$-cyclopropyl, cyclobutyl, $-CH_2$-cyclobutyl, cyclopentyl, $-CH_2$-cyclopentyl-n, cyclohexyl, $-CH_2$-cyclohexyl, cyclohexenylethyl, cyclohexanylethyl, norborbyl moieties and the like, which again, may bear one or more substituents.

[0022] The term "alkoxy" (or "alkyloxy"), or "thioalkyl" as used herein refers to an alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom or through a sulfur atom. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

[0023] The term "alkylamino" refers to a group having the structure -NHR'wherein R' is alkyl, as defined herein. The term "aminoalkyl" refers to a group having the structure NH_2R'-, wherein R' is alkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group

contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.

[0024] Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0025] In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. It will also be appreciated that aryl and heteroaryl moieties, as defined herein may be attached via an aliphatic, alicyclic, heteroaliphatic, heteroalicyclic, alkyl or heteroalkyl moiety and thus also include –(aliphatic)aryl, -(heteroaliphatic)aryl, -(aliphatic)heteroaryl, -(heteroaliphatic)heteroaryl, –(alkyl)aryl, -(heteroalkyl)aryl, -(heteroalkyl)aryl, and –(heteroalkyl)heteroaryl moieties. Thus, as used herein, the phrases "aryl or heteroaryl" and "aryl, heteroaryl, –(aliphatic)aryl, -(heteroaliphatic)aryl, -(aliphatic)heteroaryl, -(heteroaliphatic)heteroaryl, –(alkyl)aryl, -(heteroalkyl)aryl, -(heteroalkyl)aryl, and –(heteroalkyl)heteroaryl" are interchangeable. Substituents include, but are not limited to, any of the previously mentioned substitutents, *i.e.*, the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present invention, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. In certain

embodiments of the present invention, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

[0026] It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one, two or three of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0027] The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of aliphatic, heteroaliphatic or heterocyclic moieties, may optionally be substituted with substituents including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x

independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0028] The term "heteroaliphatic", as used herein, refers to aliphatic moieties in which one or more carbon atoms in the main chain have been substituted with a heteroatom. Thus, a heteroaliphatic group refers to an aliphatic chain which contains one or more oxygen, sulfur, nitrogen, phosphorus or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be branched or linear unbranched. In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to aliphatic; alicyclic; heteroaliphatic; heteroalicyclic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heteroalicyclic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0029] The term "heteroalicyclic", as used herein, refers to compounds which combine the properties of heteroaliphatic and cyclic compounds and include but are not limited to saturated and unsaturated mono- or polycyclic heterocycles such as morpholino, pyrrolidinyl, furanyl, thiofuranyl, pyrrolyl etc., which are optionally substituted with one or more functional groups, as defined herein.

[0030] Additionally, it will be appreciated that any of the alicyclic or heteroalicyclic moieties described above and herein may comprise an aryl or heteroaryl moiety fused thereto. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0031] The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

[0032] The term "haloalkyl" denotes an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

[0033] The term "heterocycloalkyl" or "heterocycle", as used herein, refers to a non-aromatic 5-, 6- or 7- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. Representative heterocycles include, but are not limited to, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and tetrahydrofuryl. In certain embodiments, a "substituted heterocycloalkyl or heterocycle" group is utilized and as used herein, refers to a heterocycloalkyl or heterocycle group, as defined above, substituted by the independent replacement of one, two or three of the hydrogen atoms thereon with but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substitutents described above and herein may be substituted or

unsubstituted. Additional examples or generally applicable substituents are illustrated by the specific embodiments shown in the Examples, which are described herein.

[0034] As used herein, the terms "aliphatic", "heteroaliphatic", "alkyl", "alkenyl", "alkynyl", "heteroalkyl", "heteroalkenyl", "heteroalkynyl", and the like encompass substituted and unsubstituted, saturated and unsaturated, and linear and branched groups. Similarly, the terms "alicyclic", "heteroalicyclic", "heterocycloalkyl", "heterocycle" and the like encompass substituted and unsubstituted, and saturated and unsaturated groups. Additionally, the terms "cycloalkyl", "cycloalkenyl", "cycloalkynyl", "heterocycloalkyl", "heterocycloalkenyl", "heterocycloalkynyl", "aryl", "heteroaryl" and the like encompass both substituted and unsubstituted groups.

[0035] It will be appreciated that additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples which are described herein, but are not limited to these Examples.

[0036] The phrase, "pharmaceutically acceptable derivative", as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof. Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety, which is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester, which is cleaved *in vivo* to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention. Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives will be discussed in more detail herein below.

[0037] By the term "protecting group", has used herein, it is meant that a particular functional moiety, *e.g.*, O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the other functional groups; the

protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen and carbon protecting groups may be utilized. For example, in certain embodiments, as detailed herein, certain exemplary oxygen protecting groups are utilized. These oxygen protecting groups include, but are not limited to methyl ethers, substituted methyl ethers (*e.g.*, MOM (methoxymethyl ether), MTM (methylthiomethyl ether), BOM (benzyloxymethyl ether), PMBM or MPM (p-methoxybenzyloxymethyl ether), to name a few), substituted ethyl ethers, substituted benzyl ethers, silyl ethers (*e.g.*, TMS (trimethylsilyl ether), TES (triethylsilylether), TIPS (triisopropylsilyl ether), TBDMS (t-butyldimethylsilyl ether), tribenzyl silyl ether, TBDPS (t-butyldiphenyl silyl ether), to name a few), esters (*e.g.*, formate, acetate, benzoate (Bz), trifluoroacetate, dichloroacetate, to name a few), carbonates, cyclic acetals and ketals. In certain other exemplary embodiments, nitrogen protecting groups are utilized. These nitrogen protecting groups include, but are not limited to, carbamates (including methyl, ethyl and substituted ethyl carbamates (*e.g.*, Troc), to name a few) amides, cyclic imide derivatives, N-Alkyl and N-Aryl amines, imine derivatives, and enamine derivatives, to name a few. Certain other exemplary protecting groups are detailed herein, however, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the present invention. Additionally, a variety of protecting groups are described in "Protective Groups in Organic Synthesis" Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

[0038] As used herein, the term "adjuvant" or "immunogenic stimulant" refers to a moiety, which, when co-administered with an immunogen, enhances the immunogenicity of the immunogen. Specifically, in certain embodiments, immunogenicity of the inventive GP120 compounds can be significantly improved if the immunizing agent(s) (*e.g.*, GP120 glycan and/or glycopeptide(s)) and/or composition thereof is, regardless of administration format, co-immunized with an adjuvant. Commonly, adjuvants are used as an 0.05 to 1.0 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an immunogen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the immunogen locally

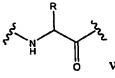
near the site of administration to produce a depot effect facilitating a slow, sustained release of immunogen to cells of the immune system. Adjuvants can also attract cells of the immune system to an immunogen depot and stimulate such cells to elicit immune responses. As such, embodiments of this invention encompass compositions further comprising adjuvants.

[0039] Adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants (such as lipopolysaccharides) normally are the components of killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects making them unsuitable for use in humans and many animals. Indeed, aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established. Notwithstanding, it does have limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response with other immunogens. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents. In addition to adjuvants used for therapeutic purposes (e.g., vaccines), other adjuvants may be used for raising antibodies in animals, which antibodies may be used, for example, in diagnostic and immunoassays. Examples of such adjuvants include, but are not limited to, bacteria or liposomes. For example, suitable adjuvants include but are not limited to, saponin adjuvants (*e.g.*, GPI-0100), *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

[0040] A wide range of extrinsic adjuvants can provoke potent immune responses to immunogens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

[0041] The term "natural amino acid" as used herein refers to any one of the common, naturally occurring L-amino acids found in naturally occurring proteins: glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), lysine (Lys), arginine (Arg), histidine (His),

proline (Pro), serine (Ser), threonine (Thr), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamine (Gln), cysteine (Cys) and methionine (Met).

[0042] The term "unnatural amino acid" as used herein refers to all amino acids which are not natural amino acids. This includes, for example, α-, β-, D-, L- amino acid residues, and compounds of the general formula  wherein the side chain R is other than the amino acid side chains occurring in nature.

[0043] More generally, the term "amino acid", as used herein, encompasses natural amino acids and unnatural amino acids.

[0044] As used herein the term "*biological sample*" includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from an animal (*e.g.*, mammal) or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof. For example, the term "biological sample" refers to any solid or fluid sample obtained from, excreted by or secreted by any living organism, including single-celled micro-organisms (such as bacteria and yeasts) and multicellular organisms (such as plants and animals, for instance a vertebrate or a mammal, and in particular a healthy or apparently healthy human subject or a human patient affected by a condition or disease to be diagnosed or investigated). The biological sample can be in any form, including a solid material such as a tissue, cells, a cell pellet, a cell extract, cell homogenates, or cell fractions; or a biopsy, or a biological fluid. The biological fluid may be obtained from any site (*e.g.* blood, saliva (or a mouth wash containing buccal cells), tears, plasma, serum, urine, bile, seminal fluid, cerebrospinal fluid, amniotic fluid, peritoneal fluid, and pleural fluid, or cells therefrom, aqueous or vitreous humor, or any bodily secretion), a transudate, an exudate (*e.g.* fluid obtained from an abscess or any other site of infection or inflammation), or fluid obtained from a joint (*e.g.* a normal joint or a joint affected by disease such as rheumatoid arthritis, osteoarthritis, gout or septic arthritis). The biological sample can be obtained from any organ or tissue (including a biopsy or autopsy specimen) or may comprise cells (whether primary cells or cultured cells) or medium conditioned by any cell, tissue or organ. In certain embodiments, the biological sample is obtained from the prostate epithelium. Biological samples may also include sections of tissues such as frozen sections taken for

histological purposes. Biological samples also include mixtures of biological molecules including proteins, lipids, carbohydrates and nucleic acids generated by partial or complete fractionation of cell or tissue homogenates. Although the sample is preferably taken from a human subject, biological samples may be from any animal, plant, bacteria, virus, yeast, etc. The term *animal*, as used herein, refers to humans as well as non-human animals, at any stage of development, including, for example, mammals, birds, reptiles, amphibians, fish, worms and single cells. Cell cultures and live tissue samples are considered to be pluralities of animals. In certain exemplary embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). An animal may be a transgenic animal or a human clone. If desired, the biological sample may be subjected to preliminary processing, including preliminary separation techniques. In certain emboidments, the biological sample is taken from a male human subject.

[0045] As used herein, the term "isolated" when applied to the compounds of the present invention refers to such compounds when removed from their naturally occurring location, that are not substantially contaminated with, or otherwise in contact with any other compound. Accordingly, the present invention provides compounds of formula **(I)** and/or **(II)** in substantially pure form, *i.e.*, in a purity of greater than about 95% by weight, preferably greater than about 98%, and more preferably greater than about 99% by weight. In one aspect, the impurity in contact with a compound of formula **(I)** and/or **(II)** of the invention is an organic chemical, *e.g.*, an organic solvent. In another aspect, the impurity in contact with a compound of formula **(I)** and/or **(II)** is another compound of formula **(I)** and/or **(II)**. Thus, in one aspect, the present invention provides a compound of formula **(I)** and/or **(II)** that is pure in that it is not in contact with another compound of formula **(I)** and/or **(II)**.

[0046] As used herein, the term "GP120 glycan" refers to a carbohydrate domain expressed in GP120. More specifically, GP120 glycan designates the carbohydrate portion of compounds of formula **(I)**, **(II)** and/or **(III)** described herein. In certain embodiments, the term refers to compounds of formula **(I)**, **(II)** and/or **(III)** where R^4 is a moiety other than a peptide, protein or other polymeric construct.

[0047] As used herein, the term "GP120 glycopeptide" refers to compounds of formula **(I)**, **(II)** and/or **(III)** where R^4 comprises a peptide moiety covalently linked to the rest of the construct either directly (*e.g.*, through N or O) or through a crosslinker.

[0048] As used herein, the term "eliciting an immune response" is defined as initiating, triggering, causing, enhancing, improving or augmenting any response of the immune system, for example, of either a humoral nature. The initiation or enhancement of an immune response can be assessed using assays known to those skilled in the art including, but not limited to, antibody assays (for example ELISA assays). In certain exemplary embodiments, the GP120 glycans and glycopeptides of the present invention, and the method of the present invention essentially trigger or enhance a humoral immune response.

BRIEF DESCRIPTION OF THE DRAWING

[0049] Figure 1 depicts structures of GP120 glycopeptides **1-2**.

DETAILED DESCRIPTION OF CERTAIN PREFERRED EMBODIMENTS OF THE INVENTION

[0050] As discussed above, the desire to develop improved methods for the preparation of synthetic vaccines has led to increased research efforts directed toward the synthesis of naturally occurring complex carbohydrate antigens, as well as novel complex structures (*e.g.*, glycopeptides) incorporating these antigenic structures. As is often the case during the course of any such large synthetic undertaking, improved synthetic methods are often developed that can be applied universally. In particular, synthetic studies of naturally occurring antigenic structures has led to the development of novel methodologies enabling the development of heretofore unavailable synthetic carbohydrate-based vaccines. For a review, see Danishefsky, S.J.; Allen, J.R., *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 836-863, and references cited therein.

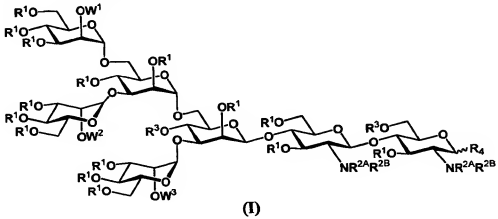
[0051] Significantly, the present invention provides novel methodologies for the synthesis of complex carbohydrates and related therapeutic compounds (*e.g.*, glycoconjugates and/or glycopeptides). In particular, in the context of synthetic studies developed for the total synthesis of glycosylated fragments of gp120 and conjugates thereof, generalized methodologies were developed for the improved synthesis of complex carbohydrate structures. This general synthetic method encompasses the realization that the incorporation of an amino group at the reducing end of a carbohydrate acceptor allows for accessibility to complex N-linked carbohydrate conjugates. In yet another aspect, the present invention also provides the recognition that for certain protected carbohydrates, the amino carbohydrate moieties can serve as useful precursors that can be utilized ultimately for the synthesis of complex N-linked glycopeptides.

[0052] Specific examples, particularly with respect to the total synthesis of N-acetyllactosamine-type glycans and their incorporation into gp120 glycopeptide fragments are described in more detail below, along with certain general methodologies developed during the course of these syntheses. It will be appreciated by one of ordinary skill in the art that these examples are not intended to be limiting; rather all equivalents are intended to be incorporated into the scope of the present invention.

[0053] **1) Inventive Compounds**

[0054] As mentioned above, the total synthesis of complex antigenic structures has led to significant development in methodologies for complex carbohydrate synthesis. Of particular recent interest is the naturally occurring antigenic gp120 glycans; e.g., "high-mannose"- and "hybrid"-type glycoforms thereof (See constructs **1-2** in Figure 1) which heretofore had not yet been synthesized.

[0055] Thus, in one aspect of the present invention, the synthesis of the complex GP120 carbohydrate domains has been achieved and an isolated compound of formula (**I**) having the structure as shown below is provided:



(**I**)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

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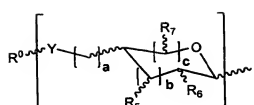
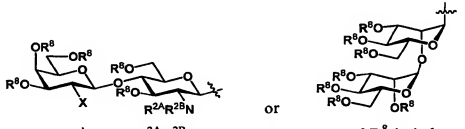


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties;

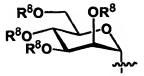
and wherein R^4 is $-OR^{4A}$ or $-NHR^{4A}$; wherein R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N or O atom to which it is attached, either directly or through a crosslinker.

[0056] In certain embodiments, W^3 is R^1, R^3, as defined above, or a moiety having the structure:



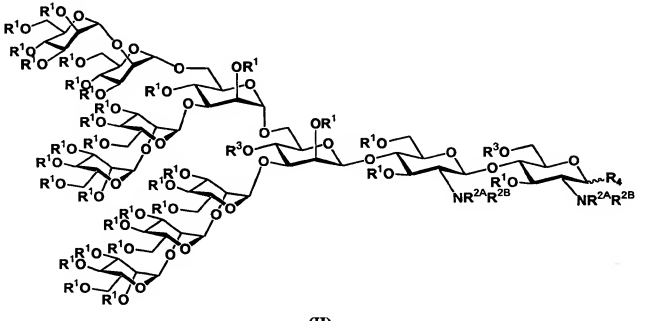
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

[0057] In certain embodiments, W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:



wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

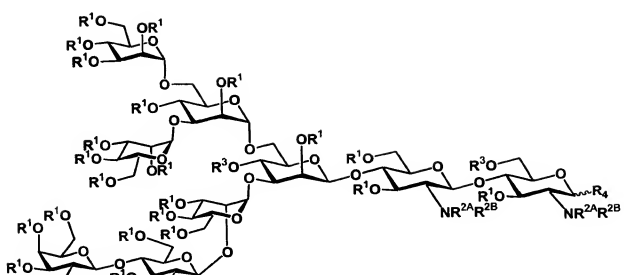
[0058] In certain embodiments, a compound of formula **(II)** having the structure as shown below is provided:



(II)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined above.

[0059] In certain embodiments, a compound of formula **(III)** having the structure as shown below is provided:

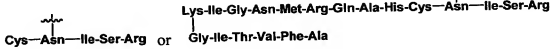


(III)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined above and X is OR^1 or $NR^{2A}R^{2B}$.

[0060] In certain embodiments, compounds of formula **(I)**, **(II)** or **(III)** exclude naturally occurring gp120 (e.g., a glycan fragment of naturally occurring gp120 glycoprotein).

[0061] In certain embodiments, when R^4 comprises a peptide, the peptide is either identical to or closely related to that of gp120 near an N-glycosylation site. In certain exemplary embodiments, the peptide has the structure:



or truncated, elongated or derivatized version thereof; wherein any one or more of the amino acid residues may bear one or more protecting groups. For the purpose of the invention, "truncated", refers to a peptide fragment comprising no fewer than about 6 amino acid residues; "elongated", refers to a peptide comprising no more than about 60 amino acid residues; and "derivatized" refers to a peptide in which at least one, but not more than about 2 out of every 10, amino acid residues have been added and/or deleted; and/or in which at least one amino acid residue has been substituted with a natural or non-natural amino acid residue so that the resulting peptide has a sequence identity equal or greater to about 70% with the original peptide.

[0062] In certain exemplary embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, each occurrence of R^1 is independently an oxygen protecting group. In certain other

exemplary embodiments, each occurrence of R^1 is independently hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, -$Si(R^{1A})_3$, -$C(=O)R^{1A}$, -$C(=S)R^{1A}$, -$C(=NR^{1A})R^{1B}$, -SO_2R^{1A}, wherein R^{1A} and R^{1B} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, -$C(=O)R^{1C}$ or–ZR^{1C}, wherein Z is –O-, -S-, -NR^{1D}, wherein each occurrence of R^{1C} and R^{1D} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety. In yet other exemplary embodiments, each occurrence of R^1 is independently hydrogen, alkylaryl, -$Si(R^{1A})_3$ or -$C(=O)R^{1A}$, wherein R^{1A} is as defined above. In yet other exemplary embodiments, each occurrence of R^1 is independently hydrogen, Bn or Bz. In certain other exemplary embodiments, each occurrence of R^1 is independently hydrogen.

[0063] In certain other exemplary embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, for each occurrence of –$NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently a nitrogen protecting group. In certain other exemplary embodiments, each occurrence of –$NR^{2A}R^{2B}$, R^{2A} and R^{2B} is independently hydrogen, alkyl, alkenyl, –$C(=O)R^{2C}$, –$C(=O)OR^{2C}$, -SR^{2C}, SO_2R^{2C}, or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; wherein each occurrence of R^{2C} is independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, -$C(=O)R^{2D}$ or–ZR^{2D}, wherein Z is –O-, -S-, -NR^{2E}, wherein each occurrence of R^{2D} and R^{2E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety. In certain exemplary embodiments, for each occurrence of –$NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently –$C(=O)R^{2A}$ or SO_2R^{2A}; or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety. In yet other exemplary embodiments, for each occurrence of –$NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B}

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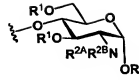
is independently $-C(=O)R^{2C}$ or SO_2R^{2C} wherein R^{2C} is as defined above, or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted phthalimide moiety. In yet other exemplary embodiments, for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently acyl, $-SO_2Ph$ or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted phthalimide moiety. In certain other exemplary embodiments, each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

[0064] In certain other embodiments, for compounds of formula **(III)** above, X is $-OR^1$, wherein R^1 is as defined generally above and in classes and subclasses herein.

[0065] In certain other embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, each occurrence of R^3 is independently R^1, wherein R^1 is as defined generally above and in classes and subclasses herein. In certain embodiments, each occurrence of R^3 is independently hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{3B}$ or–ZR^{3B}, wherein Z is –O-, -S-, $-NR^{3C}$, wherein each occurrence of R^{3B} and R^{3C} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety. In yet other exemplary embodiments, each occurrence of R^3 is independently hydrogen, Bn or Bz. In certain other exemplary embodiments, each occurrence of R^3 is independently hydrogen.

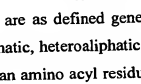
[0066] In certain other embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, each occurrence of R^1 and R^3 is independently hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is as defined above. In yet other exemplary embodiments, each occurrence of R^1 and R^3 is independently hydrogen, Bn or Bz. In certain other exemplary embodiments, each occurrence of R^1 is Bn and each occurrence of R^3 is Bz. In certain other exemplary embodiments, each occurrence of R^1 and R^3 is independently hydrogen.

[0067] In certain embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, R^4 is $-OR^{4A}$ and the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; R^{4A} is hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, $-Si(R^{4B})_3$, $-C(=O)R^{4B}$, $-C(=S)R^{4B}$, $-C(=NR^{4B})R^{4C}$, $-SO_2R^{4B}$, wherein R^{4B} and R^{4C} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{4D}$ or $-ZR^{4D}$, wherein Z is –O-, -S-, $-NR^{4E}$, wherein each occurrence of R^{4D} and R^{4E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety; or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the O atom to which it is attached, either directly or through a crosslinker. In yet other exemplary embodiments, R^{4A} is $-Si(R^{4B})_3$, wherein R^{4B} is as defined above. In yet other exemplary embodiments, R^{4A} is TBS. In yet other exemplary embodiments R^{4A} comprises a serine (ser) amino acyl residue. In yet other exemplary embodiments R^{4A} comprises a threonine (Thr) amino acyl residue. In yet other exemplary embodiments R^{4A} comprises a peptide attached to O through a serine (Ser) residue. In yet other exemplary embodiments R^{4A} comprises a peptide attached to O through a Threonine (Thr) residue.

[0068] In certain embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, R^4 is $-NHR^{4A}$ and the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; and R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein,

peptide or lipid moiety covalently linked to the rest of the construct, or to the N atom to which it is attached, either directly or through a crosslinker.

[0069] In certain exemplary embodiments, R^{4A} is hydrogen.

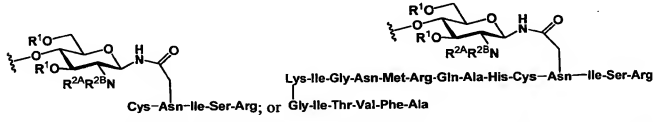
[0070] In certain other exemplary embodiments, R^{4A} comprises an amino acyl residue of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site.

[0071] In certain other exemplary embodiments, R^{4A} comprises an Asparagine residue (Asn) of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site.

[0083] For the purpose of the invention, a peptide whose structure is "closely related to that of gp120 near an N-glycosylation site" designates a gp120 peptide fragment, or truncated, elongated or derivatized version thereof, comprising $\leq$ about 60 amino acid residues, wherein one amino acid residue bears an N-glycosylation site, at least one amino acid residue has been added, deleted and/or substituted with a natural or non-natural amino acid residue, so that the resulting peptide has a sequence identity greater or equal to about 70% with the original GP120 peptide fragment. In certain embodiments, the peptide comprises $\leq$ about 55 amino acid residues. In certain embodiments, the peptide comprises $\leq$ about 50 amino acid residues. In certain embodiments, the peptide comprises $\leq$ about 45 amino acid residues. In certain embodiments, the peptide comprises $\leq$ about 40 amino acid residues. In certain embodiments, the peptide comprises $\leq$ about 35 amino acid residues. In certain embodiments, the peptide comprises $\leq$ about 30 amino acid residues. In certain embodiments, the peptide comprises $\leq$ about 25 amino acid residues. In certain embodiments, the peptide comprises $\leq$ about 20 amino acid residues. In certain embodiments, the peptide has a sequence identity greater or equal to about 75% with the original GP120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 80% with the original GP120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 85% with the original GP120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 90% with the original GP120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 95% with the original GP120 peptide fragment.

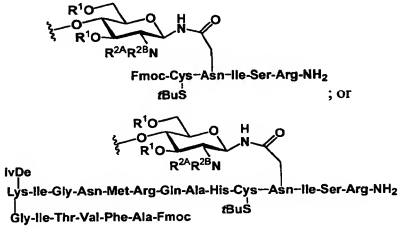
[0084] A peptide whose structure is "identical to that of GP120 near an N-glycosylation site" designates a GP120 peptide fragment of a naturally occurring GP120 glycoprotein, comprising ≤ about 60 amino acid residues, wherein one amino acid residue bears an N-glycosylation site. In certain embodiments, the peptide comprises ≤ about 55 amino acid residues. In certain embodiments, the peptide comprises ≤ about 50 amino acid residues. In certain embodiments, the peptide comprises ≤ about 45 amino acid residues. In certain embodiments, the peptide comprises ≤ about 40 amino acid residues. In certain embodiments, the peptide comprises ≤ about 35 amino acid residues. In certain embodiments, the peptide comprises ≤ about 30 amino acid residues. In certain embodiments, the peptide comprises ≤ about 25 amino acid residues. In certain embodiments, the peptide comprises ≤ about 20 amino acid residues.

[0085] In certain embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, R^4 is $-NHR^{4A}$ wherein R^{4A} comprises an Asparagine residue (Asn) of a peptide whose structure is either identical or closely related to that of GP120 near an N-glycosylation site and the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein and wherein any of the amino acid residues may bear one or more protecting groups.

[0086] In certain exemplary embodiments, the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein.

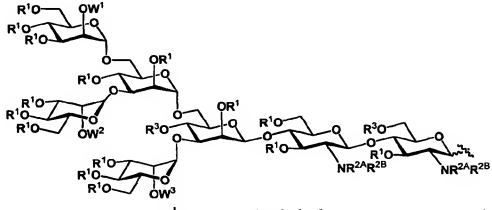
[0087] In certain other exemplary embodiments, the saccharide unit bearing R^4 has the structure:

Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys—Asn-Ile-Ser-Arg-NH_2

Gly-Ile-Thr-Val-Phe-Ala-NH_2

wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein.

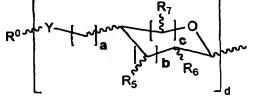
[0077] In certain embodiments, the invention provides constructs comprising one or more carbohydrate domains expressed on the surface of gp120. In certain embodiments, there is provided an antigenic construct comprising one or more carbohydrate domains having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

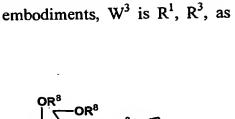


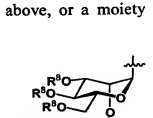
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties

wherein each carbohydrate domain is independently covalently bound to a linker system, said linker system being a peptide or non-peptide nature.

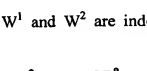
[0078] In certain embodiments, W^3 is R^1, R^3, as defined above, or a moiety having the structure:



or


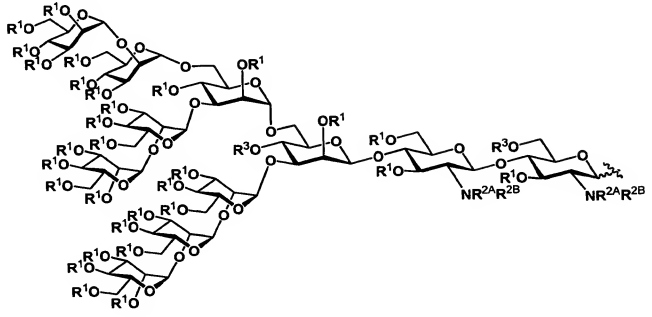
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

[0079] In certain other embodiments, W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:

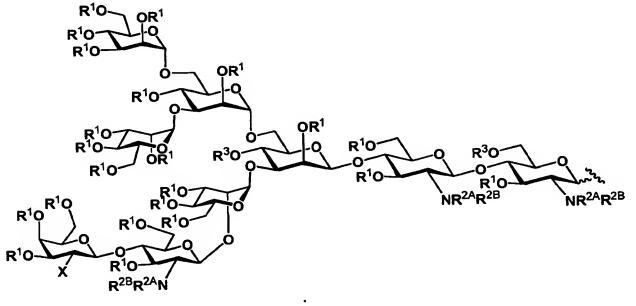


wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

[0080] In yet other embodiments, inventive constructs comprise one or more carbohydrate domains having the structure:



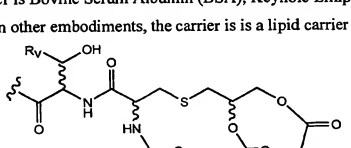
[0081] In yet other embodiments, inventive constructs comprise one or more carbohydrate domains having the structure:



[0082] In certain embodiments, some or all of carbohydrate domains are O-linked to the linker system. In certain other embodiments, some or all of carbohydrate domains are N-linked to the linker system. In yet other embodiments, the linker system is a peptide. In certain

embodiments, the linker system is a peptide and comprises two or more carbohydate domains covalently attached thereto, wherein the peptide sequence between each point of attachment of the carbohydrate domains comprises a cysteine residue. In certain embodiments, the mutliglycan construct is prepared by Native Chemical Ligation. In certain embodiments, the inventive constructs are symmetrical, nonsymmetrical and mixed (N-linked and O-linked carbohydrates). In certain embodiments, the linker system is designed to approximate the spatial position(s) of carbohydrate(s) in gp120. In yet other embodiments, the linker system is further attached to a carrier immunostimulant.

[0083] In certain embodiments, any of the isolated compounds, glycopeptides and/or constructs described herein may be further conjugated to an immunogenic carrier. In certain exemplary embodiments, the carrier is a protein, a peptide or a lipid. In certain other exemplary embodiments, the carrier is Bovine Serum Albumin (BSA), Keyhole Limpet Hemocyanin (KLH) or polylysine. In certain other embodiments, the carrier is is a lipid carrier having the structure:

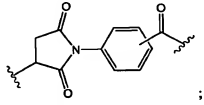


wherein m, n and p are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys).

[0084] It will be appreciated that the carrier can be linked to the rest of the construct either directly or through a crosslinker, and thus R^4 encompasses proteins, peptides, and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties.

[0085] Crosslinkers suited to the invention are widely known in the art (see, for example, 1994 Pierce Technical Handbook: cross-linking (Appendix A), which is available at http://www.piercenet.com/ resources/browse.cfm?fldID=184), including bromoacetic NHS ester, 6-(iodoacetamido)caproic acid NHS ester, maleimidoacetic acid NHS ester, maleimidobenzoic acide NHS ester, etc. In certain preferred embodiments, the crosslinker is MMCCH (4-

(maleimidomethyl) cyclohexane-1-carboxyl hydrazide). In certain other preferred embodiments, the crosslinker is MBS (m-maleimidobenzoyl acid N-Hydroxysuccinimidyl ester). In certain embodiments, the crosslinker is a fragment having the structure:



;

whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a suitable functionality on R^4.

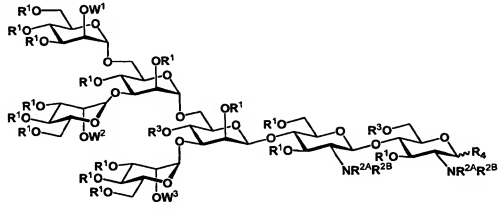
[0086] 2) Synthetic Methodology

[0087] The practitioner has a a well-established literature of carbohydrate chemistry to draw upon, in combination with the information contained herein, for guidance on synthetic strategies, protecting groups, and other materials and methods useful for the synthesis of the compounds of this invention, and conjugates thereof.

[0088] The various patent documents and other references cited herein provide helpful background information on preparing compounds similar to the inventive compounds described herein or relevant intermediates, as well as information on formulation, uses, and administration of such compounds which may be of interest.

[0089] Moreover, the practitioner is directed to the specific guidance and examples provided in this document relating to various exemplary compounds and intermediates thereof.

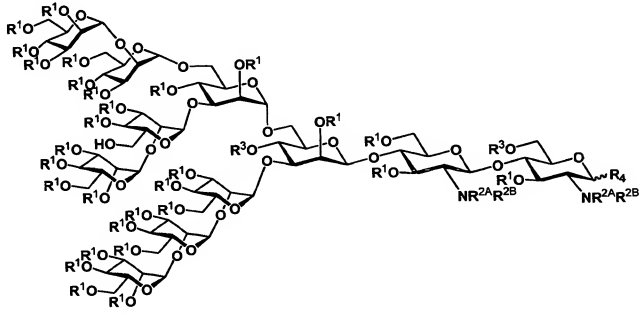
[0090] In one aspect of the invention, there is provided a method for preparing isolated an compound of formula (**I**):



(I)

[0091] wherein R^1, R^{2A}, R^{2B}, R^3, R^4 and W^1-W^3 are as defined generally above and in classes and subclasses herein.

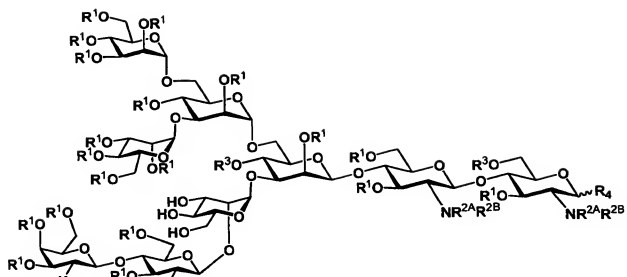
[0092] In one aspect of the invention, there is provided a method for preparing isolated an compound of formula **(II)**:



(II)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined generally above and in classes and subclasses herein.

[0093] In another aspect of the invention, there is provided a method for preparing isolated an compound of formula **(III)**:



(III)

[0094] wherein X, R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined generally above and in classes and subclasses herein.

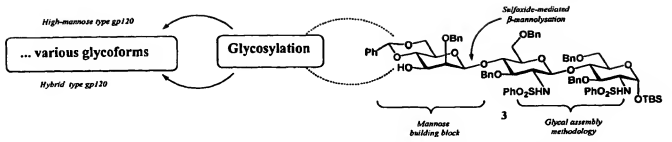
[0095] In certain exemplary embodiments, R^4 is $-NHR^{4A}$; wherein R^{4A} is an amino acyl residue of a peptide and the invention provides a method for preparing homogeneous N-linked GP120-derived glycopeptides.

[0096] ***Glycan Synthesis***

[0097] Glycan synthesis generally suffers from the stereochemical diversity of its targets and therefore of its building blocks, as well. The advent of a new target often requires a reworked, if not entirely different synthetic plan, based on varying protecting groups, coupling strategies, and starting materials. The present invention provides a method allowing access to a number of GP120-derived saccharides using only a small set of building blocks and the same general procedure for each glycan.

[0098] In certain embodiments, trisaccharide **3** in Scheme 1 embodies the protected core structure reported for the glycoforms expressed in GP120.

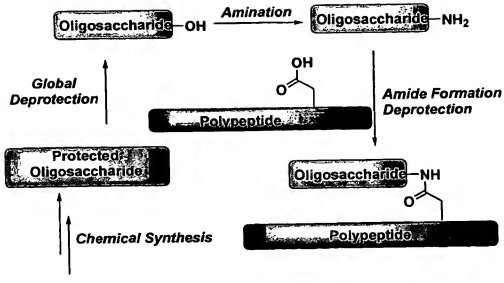
[0099] **Scheme 1.** Proposed methodology for glycan synthesis.



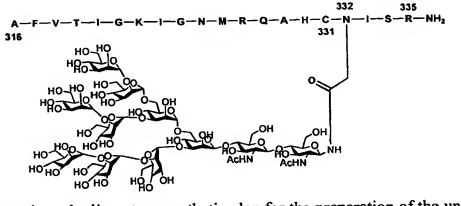
[0100] In certain exemplary embodiments, trisaccharide **3** may be elaborated to give a pentasaccharide either by deprotection of the 6-position followed by simultaneous α-mannosylation at the free 3- and 6-positions or by sequential mannosylation at the 3-and 6-positions with an intermediate deprotection step. Simultaneous mannosylation with equivalently protected mannosyl donors would yield a "symmetrically" substituted pentasaccharide; further deprotections and glycosylations could be achieved in a synchronous fashion at both nonreducing termini. Sequential mannosylation would allow the inclusion of differentially protected mannose building blocks, permitting independent elaboration of the 3- and 6-substituted antennae. Thus the high-mannose pentasaccharide core (which is conserved in most natural *N*-linked glycans) may be synthesized in large quantities and used as a starting point for all of the GP120 targets. Moreover, because hybrid-type GP120 differs from high-mannose type GP120 in its degree of branching beyond the core pentasaccharide, this synthetic scheme would provide easy access to the multi-antennary glycoforms expressed in GP120.

[0101] In certain embodiments, the synthetic approach includes: synthesis of protected oligasaccaride (undecassaccharide), global deprotection to prepare free glycan, amination, coupling with peptide acid and deprotection (Scheme 2).

[0102] **Scheme 2.** Exemplary synthetic strategy

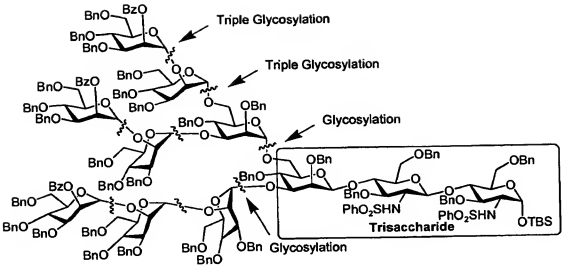


[0103] In certain embodiments, a synthesis for the high-mannose type glycopeptide having the structure:



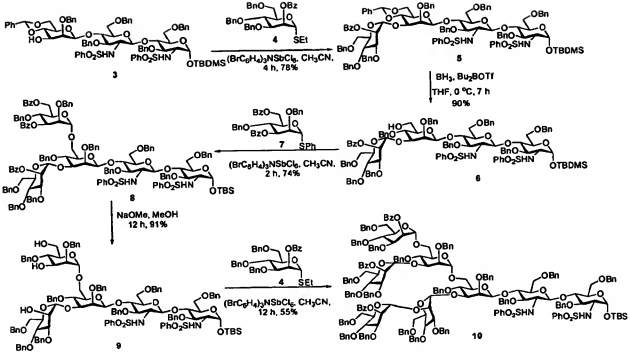
is provided. In certain embodiments, a synthetic plan for the preparation of the undecasaccharide is shown in Scheme 3. For example, starting from a trisaccharide intermediate (e.g., trisacchraide **3**),[1] two successive glycosylations will give pentasaccharide, then two consecutive triple glycosylation would furnish the undecasaccharide.

[0104] **Scheme 3.** Exemplary retrosynthesis of undesaccharide **1**.

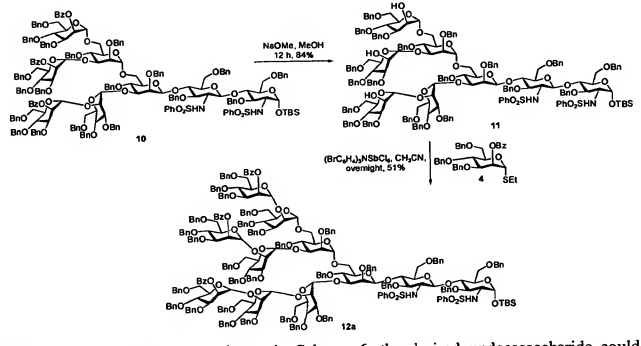


[0105] An exemplary synthesis using this route is shown in Scheme 4. For example, mannosylation of trisaccharide **3** using thiomannoside **4** and Sinaÿ radical cation activation[2,3] gave tetresaccharide in 78% yield. The benzylidene ring was reductively opened by borane and the resulting free alcohol **5** underwent mannosylation to give pentasaccharide **8** in 74% yield. After Zemplen reaction, the newly generated three free OH were mannosylated to afford octasaccharide **10** using same Sinaÿ conditions[2,3]. The same triple-glycosylation sequence was repeated to synthesize the undecsaccharide **12a** in 55% yield (Scheme 5).

[0106] **Scheme 4**



[0107] **Scheme 5**



[0108] For example, as shown in Scheme 6, the desired undecasaccharide could be synthesized by a 3+3 glycosylation (trisaccharide couples with another trisaccharide) followed by a 6+5 coupling. This synthetic plan is much shorter and more convergent than the first strategy.

Express Mail No.: EV 124826252 US
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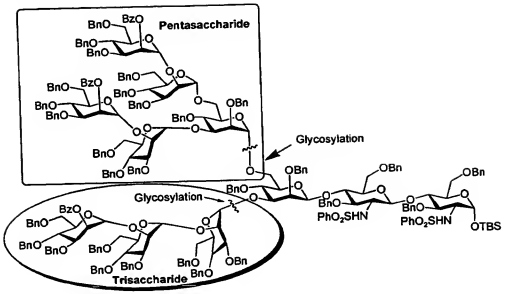
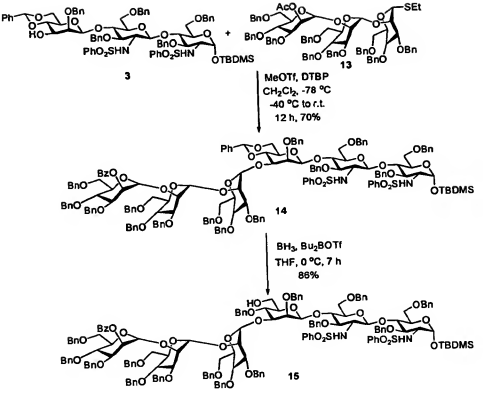
Attorney Docket No.: 2003080-0134
Client Reference No.: SK-1062-PRO3

[0109] **Scheme 6**



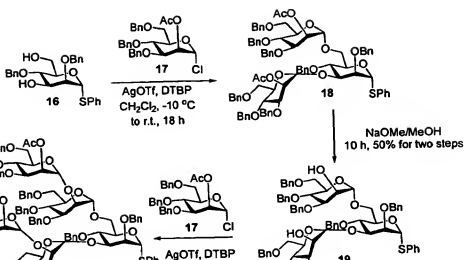
[0110] In certain embodiments, as shown in scheme 7, trisaccharide **3** first underwent glycosylation with trisaccharide donor **13** using MeOTf as promoter to afford hexasaccharide in 70% yield. Then reductive ring-opening of the benzylidene ring gave saccharide **15** in 87% yield.

[0111] **Scheme 7**



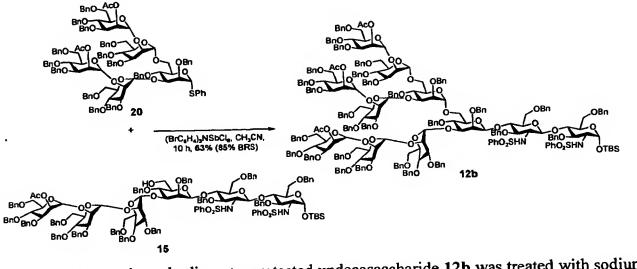
[0112] In certain embodiments, the pentasaccharide which is the precursor for the upper-left portion of the final compound **(1)** was synthesized as shown in scheme 8. For example, double-glycosylation of mannose derivative **16** using chloro donor **17** and promoter silver triflate gave trisaccharide **18**. After cleavage of the two acetyl groups, another double-glycosylation provided pentasaccharide **20** in 87% yield.

[0113] **Scheme 8**



[0114] In certain embodiments, the 6+5 glycosylation using Sinaÿ radical cation activation[2,3] proceeded smoothly giving the desired undecasaccharide **12b** in 85% yield (Scheme 9).

[0115] **Scheme 9**



[0116] In certain embodiments, protected undecasaccharide **12b** was treated with sodium methoxide and HF-pyridine to remove the acetyl groups and TBS group, respectively. The resulting oligosaccharide **21** was then subjected to global Birch deprotection followed by selective acetylation using acetyl anhydride in saturated sodium bicarbonate solution to give free glycan in high yield.[5] Following Kochetkov amination[6] furnished free glycosylamine (Scheme 10).

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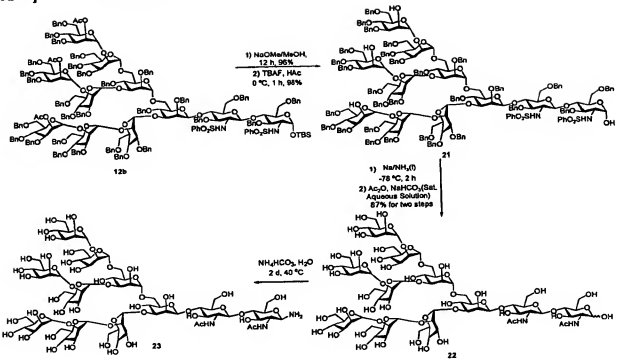
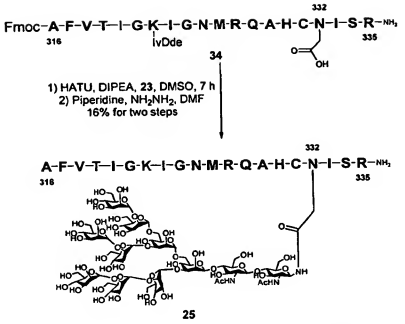
Client Reference No.: SK-1062-PRO3

[0117] **Scheme 10**



[0118] In certain embodiments, 20-mer peptide acid **34**, which was made through applied biosynthesis synthesizer, was activate using HATU and coupled directly with glycosylamine **23**. The Fomc and ivDde protecting groups were removed by treatment with hydrazine and piperidine to give glycopeptide fragment **25** in 16% two steps yield (Scheme 11).

[0119] **Scheme 11**



[0120] Methods of preparing trisaccharide 3 are known in the art. For example, guidance may be found in U.S. Provisional Patent Application No.: 60/_____entitled "Prostate Specific Antigens, Conjugates Thereof, Methods for their Preparation and Uses Thereof", filed September 4, 2003; the entire contents of which are hereby incorporated by reference herein.

[0121] It will be appreciated that natural GP120 glycosides may be sialylated and /or fucosylated to varying degrees; these saccharide residues can pose significant synthetic challenges, though they can of course be included in a glycoside synthesis (See, for example, (1) Schwarz, J. B.; Kuduk, S. D.; Chen, X. T.; Sames, D.; Glunz, P. W.; Danishefsky, S. J. "A broadly applicable method for the efficient synthesis of alpha-O-linked glycopeptides and clustered sialic acid residues." *J. Am. Chem. Soc.* **1999**, *121*, 2662-2673; and (2) Jain, R. K.; Piskorz, C. F.; Huang, B. G.; Locke, R. D.; Han, H. L.; Koenig, A.; Varki, A.; Matta, K. L. "Inhibition of L- and P-selectin by a rationally synthesized novel core 2-like branched structure containing GalNAc-Lewis(X) and Neu5Ac alpha 2-3Gal beta 1-3GalNAc sequences." *Glycobiology* **1998**, *8*, 707-717]. In certain embodiments, to circumvent this issue, rather than providing methods to synthesize (and raise antibodies against) a number of sialylated and fucosylated variants, the present invention provides a method to synthesize the most abundant glycoforms (and to generate antibodies using it). Without wishing to be bound to any particular theory, it is proposed that predigestion of a biological sample (e.g., serum) with sialidase and

fucosidase would provide a much more homogeneous sample (with respect to GP120 glycans) for immunoassay.

[0122] **References** ("Glycan synthesis" section)

[0123] **1.** Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. Tetrahedron Letters **2003**, 44, 1791-1793.

[0124] **2.** Zhang, Y.-M.; Mallet, J.-M.; Sinay, P. *Carbohydrate Research* **1992**, *236*, 73-88.

[0125] **3.** Marra, A.; Mallet, J. M.; Amatore, C.; Sinay, P. *Synlett* **1990**, 572-574.

[0126] **4.** Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *Journal of the American Chemical Society* **2003**, *125*, 3402-3403.

[0127] **5.** Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science (Washington, DC, United States)* **2003**, *300*, 2065-2071.

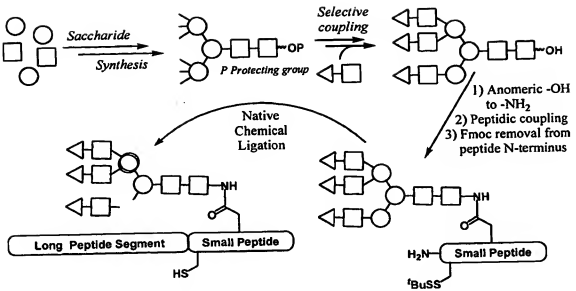
[0128] **6.** Likhosherstov, L. M.; Novikova, O. S.; Derevitskaya, V. A.; Kochetkov, N. K. *Carbohydrate Research* **1986**, *146*, C1-C5.

[0129] ***Glycopeptides***

[0130] Automated peptide synthesis is reliable for sequences up to about 60 amino acid residues in length, but saccharide moieties contained in glycopeptides render their solid phase synthesis less practical. Unlike peptide synthesis, complex glycan and glycoconjugate synthesis remains readily accessible only to a few select laboratories (See, for example, Hang, H. C.; Bertozzi, C. R. "Chemoselective approaches to glycoprotein assembly." *Acc. Chem. Res.* **2001**, *34*, 727-736). Syntheses of several natural *O*-linked glycopeptides containing simple glycans have been reported (See, for example, (1) Arsequell, G.; Haurum, J. S.; Elliott, T.; Dwek, R. A.; Lellouch, A. C. "Synthesis of Major Histocompatibility Complex Class-I Binding Glycopeptides." *J. Chem. Soc.-Perkin Trans. 1* **1995**, 1739-1745, (2) Chen, X. T.; Sames, D.; Danishefsky, S. J. "Exploration of modalities in building alpha-O-linked systems through glycal assembly: A total synthesis of the mucin-related F1 alpha antigen." *J. Am. Chem. Soc.* **1998**, *120*, 7760-7769; (3) Macmillan, D.; Bertozzi, C. R. "New directions in glycoprotein engineering." *Tetrahedron* **2000**, *56*, 9515-9525; (4) Koeller, K. M.; Smith, M. E. B.; Huang, R. F.; Wong, C.

H. "Chemoenzymatic synthesis of a PSGL- 1 N-terminal glycopeptide containing tyrosine sulfate and alpha-O-linked sialyl Lewis X." *J. Am. Chem. Soc.* **2000**, *122*, 4241-4242; (5) Ajisaka, K.; Miyasato, M.; Ishii-Karakasa, I. "Efficient synthesis of O-linked glycopeptide by a transglycosylation using endo alpha-N-acetylgalactosaminidase from Streptomyces sp." *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1240-1243; and (6) Marcaurelle, L. A.; Mizoue, L. S.; Wilken, J.; Oldham, L.; Kent, S. B. H.; Handel, T. M.; Bertozzi, C. R. "Chemical synthesis of lymphotactin: A glycosylated chemokine with a C-terminal mucin-like domain." *Chem. Eur. J.* **2001**, *7*, 1129-1132), as have examples of mimetics for *N*-linked glycopeptides (See, for example, Hang, H. C.; Bertozzi, C. R. "Chemoselective approaches to glycoprotein assembly." *Acc. Chem. Res.* **2001**, *34*, 727-736), and a chemoenzymatic synthesis of an *N*-linked glycopeptide (See, for example, Inazu, T.; Haneda, K.; Mizuno, M. "Synthetic study on N-glycopeptides." *J. Syn. Org. Chem. Jpn.* **1998**, *56*, 210-220), but no chemical synthesis has been reported for a natural *N*-linked glycopeptide with complex glycan and peptide structure. The state of the art for chemically synthesized *N*-linked glycopeptides is exemplified by the pentadecasaccharide *N*-linked to a pentapeptide reported by Wang and coworkers, which was recognized by appropriate antibodies to the H-type blood group antigens present at the glycan nonreducing termini (See, for example, Wang, Z. G.; Zhang, X. F.; Visser, M.; Live, D.; Zatorski, A.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. "Toward fully synthetic homogeneous glycoproteins: A high mannose core containing glycopeptide carrying full H-type2 human flood group specificity." *Angew. Chem. Int. Ed.* **2001**, *40*, 1728-1732).

[0131] **Scheme 12.** Exemplary synthetic approach for the preparation of GP120 glycopeptides.



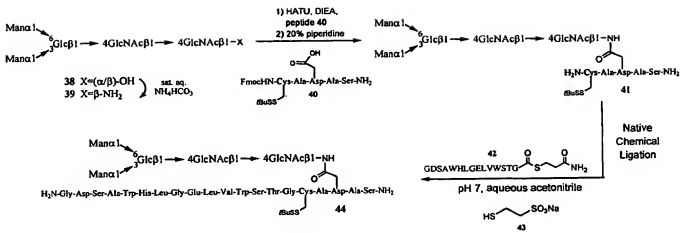
[0132] In certain embodiments, as shown in Scheme 12, the chemical synthesis of inventive glycopeptides may be divided logically into two sections: glycan synthesis (top) and glycopeptide assembly (bottom). At its core, the inventive method would extend the method of Wang, *et al.* (Wang, Z. G.; Zhang, X. F.; Visser, M.; Live, D.; Zatorski, A.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. "Toward fully synthetic homogeneous glycoproteins: A high mannose core containing glycopeptide carrying full H-type2 human flood group specificity." *Angew. Chem. Int. Ed.* **2001**, *40*, 1728-1732) to include one or more peptide elongation steps after synthesis of a short glycopeptide, allowing entry into the realm of fully elaborated, naturally derived glycoproteins (See, for example, Dawson, P. E.; Kent, S. B. H. "Synthesis of native proteins by chemical ligation." *Annu. Rev. Biochem.* **2000**, *69*, 923-960). In a subtle yet important improvement, the glycan is fashioned here in a more convergent manner than previously realized, allowing the strategy to be adjusted in its late stage to accommodate the synthesis of various glycoforms, as illustrated in the next section.

[0133] ***Glycopeptide Assembly***

[0134] Guidance for glycopeptide assembly may be found, inter alia, in U.S. Provisional Patent Application No.: 60/_____entitled "Prostate Specific Antigens, Conjugates Thereof, Methods for their Preparation and Uses Thereof", filed September 4, 2003; the entire contents of which are hereby incorporated by reference herein. For example, a glycopeptide assembly strategy, as outlined in Scheme 12, involves peptide glycosylation followed by elongation of the peptide backbone, was examined, as illustrated in Scheme 13, using a model peptide and glycan

(Miller, J. S. *et al., Angew. Chemie Int. Ed.*, 2003, **42**, 431). To prepare free glycan **38** for coupling, its anomeric hydroxyl was first aminated to give β-aminoglycoside **39** as described by Kochetkov (See, for example, Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. "A New Simple Synthesis of Amino Sugar Beta-D-Glycosylamines." *Carbohydr. Res.* **1986**, *146*, C1-C5). Glycosylamine **39** and the aspartate free acid of peptide **40** were coupled in peptidic fashion according to the procedure of Lansbury and coworkers ((1) Cohen-Anisfeld, S. T.; Lansbury, P. T. "A Practical, Convergent Method for Glycopeptide Synthesis." *J. Am. Chem. Soc.* **1993**, *115*, 10531-10537; and (2) Anisfeld, S. T.; Lansbury, P. T. "A Convergent Approach to the Chemical Synthesis of Asparagine- Linked Glycopeptides." *J. Org. Chem.* **1990**, *55*, 5560-5562) with certain modifications: the reported peptide glycosylations involved excess or equimolar amounts of glycosylamine relative to peptide, and their isolated yields (50 - 60%) are reported based on peptide starting material (Cohen-Anisfeld, S. T.; Lansbury, P. T. "A Practical, Convergent Method for Glycopeptide Synthesis." *J. Am. Chem. Soc.* **1993**, *115*, 10531-10537). As is often the case, however, the saccharide here is the more precious material entering glycosylation because its preparation involves multistep, solution phase synthesis in relatively low overall yield compared to that of the peptide. A trial glycosylation of model pentapeptide **40** with pentasaccharide **39** indicates that under the appropriate reaction conditions, an excess of peptide produces a significantly greater yield of coupled product (over 70% based on valuable glycosylamine) [Miller, J. S. *et al., Angew. Chemie Int. Ed.*, 2003, **42**, 431. Subsequent Fmoc (Fmoc = 9-fluorenylmethyloxy-carbonyl) removal with piperidine afforded glycopeptide **41**.

[0135] **Scheme 13.** Exemplary glycopeptide assembly route with a model peptide and glycan.



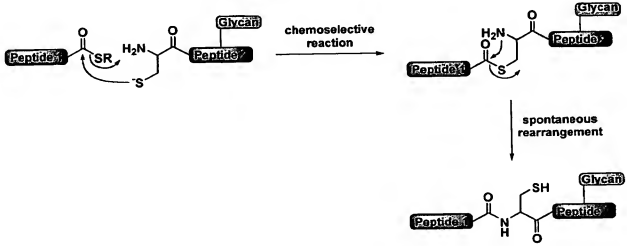
[0136] The final step toward completion of a model glycopeptide involved native chemical ligation (NCL) [See, for example, Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. "Synthesis of Proteins by Native Chemical Ligation." *Science* **1994**, *266*, 776-779], as indicated in Scheme 13. *In situ* deprotection of cysteine disulfide **41** and transthioesterification (See, for example, Dawson, P. E.; Churchill, M. J.; Ghadiri, M. R.; Kent, S. B. H. "Modulation of reactivity in native chemical ligation through the use of thiol additives." *J. Am. Chem. Soc.* **1997**, *119*, 4325-4329) of peptide thioester **42** with sodium 2-mercaptoethanesulfonate **(43)** in phosphate-buffered saline (PBS) at neutral pH led to a second thioester exchange with the (now free) cysteine thiol and subsequent rearrangement to give fully unprotected glycopeptide **44**. GP120-derived glycopeptides obtained using the strategy detailed in Scheme 13 will require no additional manipulation other than purification before they can be examined for the generation of antibodies. The synthetic strategy thus requires only four assembly steps starting from free glycans to obtain homogeneous glycopeptides.

[0137] In certain embodiments, the lysine residue is differentially protected with respect to Fmoc removal during peptide synthesis, and remains protected through the peptide glycosylation step (due to its free amine side chain). Suitably protected Lys derivatives have been designed (See, for example, Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. "An appraisal of new variants of Dde amine protecting group for solid phase peptide synthesis." *Tetrahedron Lett.* **1998**, *39*, 1603-1606), and can be deprotected in the presence of *N*-linked saccharides along with the N-terminal Fmoc amine in minutes using hydrazine at room temperature.

[0138] *Native Chemical Ligation*

[0139] One of the more widely used methods for the synthesis of glycopeptides is native chemical ligation (NCL).[i] First reported by Kent in 1994, NCL allows for the assembly of large proteins with native amide binds from unprotected peptide building blocks (Scheme 14). Furthermore, the reaction is mild, selective, and compatible with the presence of glycans. When glycans are present in the reaction, they are typically found on the *C*-terminal side. In the event, a glycopeptide containing a *C*-terminal cysteine undergoes a chemoselective reaction with a peptide thioester. The resulting peptide thioester then rearranges spontaneously to furnish a native peptide bond, effectively lengthening the peptide backbone of the glycopeptide.

[0140] **Scheme 14.** Exemplary Native Chemical Ligation methodology

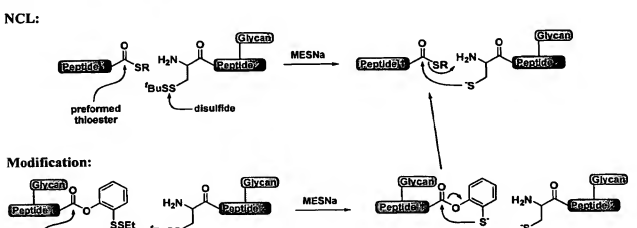


[0141] During the course of our study into the synthesis of homogeneous glycopeptides, we became interested in the generation glycopeptides bearing multiple glycosidic domains. Such structures include the synthesis of gp120 fragments, erythropoietin (EPO), human chorionic gonadotropin (HCG), and amyloid precursor protein (APP). Drawing from our experience in the area,[ii] we looked to NCL as a way to couple two small glycopeptides to form one large glycopeptide. One drawback to this method lies in the difficulty with which unprotected glycopeptide $^{\alpha}$thioesters are synthesized.[iii] Thus, if we were to be successful in our attempts to synthesize glycopeptides bearing multiple glycosidic domains, a new method for the synthesis of glycopeptide $^{\alpha}$thioesters, or their equivalent, was needed.

[0142] A closer look at the reaction mechanism of NCL provided us clues as to how this problem might be approached. In the reaction itself, the sulfhydryl group of the cysteine coupling partner, initially protected as a ^{t}butyldisulfide, is released by disulfide exchange with 2-

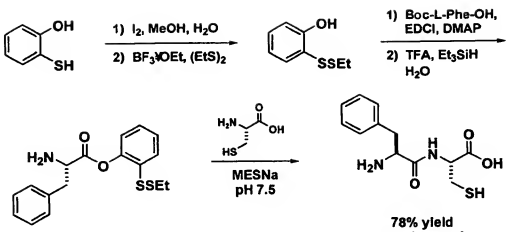
mercaptoethanesulfonic acid (MESNa) and then undergoes trans-thioesterification as mentioned above. By taking advantage of this disulfide exchange, we thought that it might be possible to mask the $^{\alpha}$thioester as a phenyl ester, bearing an ethyldisulfide in the *ortho* position (Scheme 15). Under the reaction conditions, the free sulfhydryl would be released and undergo a spontaneous rearrangement to furnish a thioester capable of entering into the NCL pathway.

[0143] **Scheme 15.** A new method for the generation of peptide $^{\alpha}$thioesters.



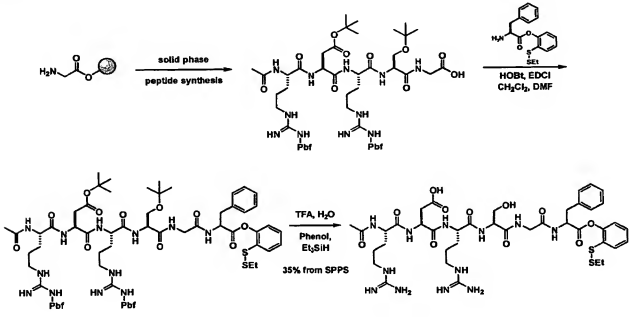
[0144] To test the viability of this proposed modification, we set out to synthesize the simple dipeptide, PheCys. The requisite *ortho*-substituted phenol is synthesized in two steps from commercially available 2-mercaptophenol by oxidation and disulfide exchange. Coupling of the phenol to phenylalanine followed by the removal of the Boc protecting group set the stage for our first attempt (Scheme 16). We were delighted to find that not only had the reaction worked, but that it had worked in good yield. More importantly, we did not observe any racemization under the reaction conditions.

[0145] **Scheme 16.** Exemplary NCL with masked thioester



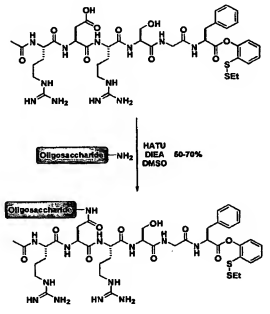
[0146] With the knowledge gained in this experiment, we turned our attention to our ultimate goal, the synthesis of glycopeptides bearing two glycosidic domains. Synthesis of the *N*-terminal fragment (the fragment that would ultimately become the masked thioester) began with the solid phase synthesis of a peptide acid, followed by standard peptide coupling to introduce the phenylalanine bearing the phenyl ester. TFA deprotection of the side chain protecting groups furnished the peptide backbone for the *N*-terminal fragment (Scheme 17).

[0147] **Scheme 17.** Exemplary synthesis of the peptide backbone of the *N*-terminal fragment.



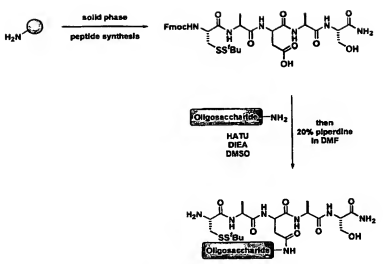
[0148] Coupling of an oligosaccharide with excess peptide furnished a fully deprotected glycopeptide ready for ligation (Scheme 18).

[0149] **Scheme 18.** Exemplary approach to the completion of the *N*-terminal fragment



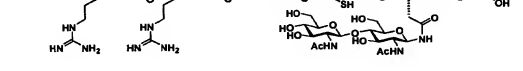
[0150] With the *N*-terminal portion in hand, we turned our attention to the synthesis of the *C*-terminal coupling partner. In a similar way, the peptide backbone was made by solid phase peptide synthesis. Upon cleavage from the resin, glycosylation and Fmoc removal, the *C*-terminal glycopeptide was obtained (Scheme 19).

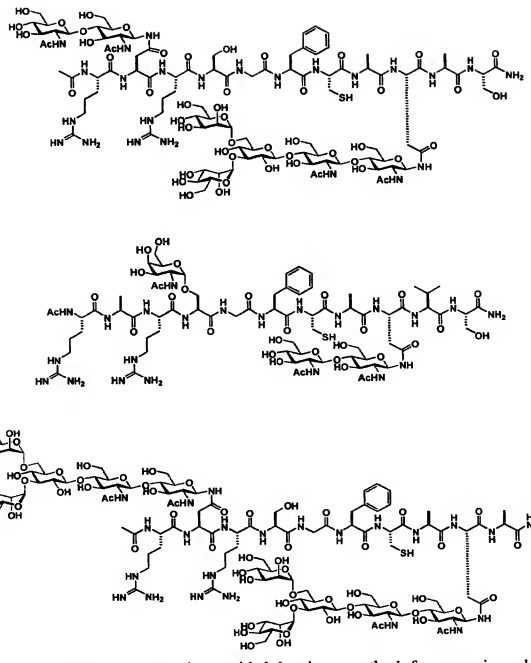
[0151] **Scheme 19.** Exemplary synthesis of the *C*-terminal glycopeptide



[0152] With the two halves in hand, we set out to determine if this new method is viable for the synthesis of glycopeptides bearing two glycosydic domains. In the event, equal molar amounts of both the *N*-terminal and *C*-terminal glycopeptides were combined in a LCMS vial and to this was added a solution of MESNa in phosphate buffered saline. The reaction was monitored by LCMS and to our amazement, the rearrangement of the phenyl ester to thioester occurred quite rapidly,[iv] additionally mass peaks corresponding to the desired product were observed almost instantly. Upon completion of the reaction, any remaining disulfide bonds were reduced by the action of tris(2-carboxyethyl) phosphine hydrochloride (TCEP). The crude reaction mixture was then subjected to HPLC for purification. We were pleased to find that the reaction had worked extremely well, providing doubly glycosylated glycopeptides, typically in 60-70% yield. Shown in Figure 1 are the glycopeptides formed to date. It should be noted that symmetrical, nonsymmetrical and mixed (*N*-linked and *O*-linked) glycopeptides can be used.

[0153] **Figure 1.** Exemplary glycopeptides formed with this method





[0154] Accordingly, there is provided herein a method for preparing glycopeptides comprising at least two carbohydate domains covalently attached thereto. In certain embodiments, some or all of carbohydrate domains are O-linked. In certain other embodiments, some or all of carbohydrate domains are N-linked. In certain embodiments, the glycopeptide comprises two or more carbohydate domains covalently attached thereto, wherein the glycopeptide sequence between each point of attachment of the carbohydrate domains comprises a cysteine residue. In certain embodiments, the mutli-glycan glycopeptide is prepared by Native

Chemical Ligation. In certain embodiments, the method allows for coupling where each coupling partner is a glycopeptide itself. Symmetrical, nonsymmetrical and mixed (N-linked and O-linked) glycopeptides can be obtained. In certain embodiments, the method involves the *in situ* generation of a thioester that is then used immediately in native chemical ligation.

[0155] **References** ("Glycopeptides" section)

[0156] **1.** a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776. b) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923. c) Grogan, M. J.; Pratt, M. R.; Marcaurelle, L. A.; Bertozzi, C. R. *Annu. Rev. Biochem.* **2002**, *71*, 593.

[0157] **2.** a) Miller, J. S.; Dudkin, V. Y.; Lyon, G. J.; Muir, T. W.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2003** *42*, 431. b) Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* Submitted for publication.

[0158] **3.** Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 11684.

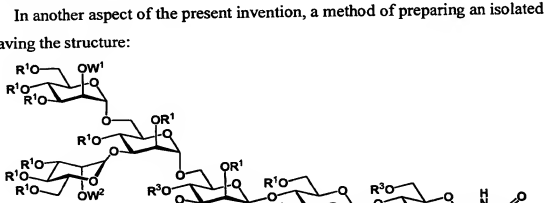
[0159] **4.** There is an intermediate step in NCL during which there is a second transthioesterification with MESNa. In our reactions we have never observed the free sulfhydryl of the phenyl ester. Rather complete conversion of the *N*-terminal to the corresponding MESNa thioester is observed within 3 min.

[0160] *Peptide Thioester Synthesis*

[0161] Several methods have been developed for peptide thioester synthesis, including the original "Boc chemistry" (Boc = *tert*-butyloxycarbonyl) method (See, for example, (1) Canne, L. E.; Walker, S. M.; Kent, S. B. H. "A General Method for the Synthesis of Thioester Resin Linkers for Use in the Solid-Phase Synthesis of Peptide Alpha-Thioacids." *Tetrahedron Lett.* **1995**, *36*, 1217-1220; and (2) Hojo, H.; Aimoto, S. "Polypeptide Synthesis Using the S-Alkyl Thioester of a Partially Protected Peptide Segment--Synthesis of the DNA- Binding Domain of C-Myb Protein (142-193)-NH2." *Bull. Chem. Soc. Jpn.* **1991**, *64*, 111-117) and several Fmoc-compatible systems (See, for example, (1) Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. "Fmoc-based synthesis of peptide-(alpha)thioesters: Application to the total chemical synthesis of a glycoprotein by native chemical ligation." *J. Am. Chem. Soc.* **1999**, *121*, 11684-11689; (2) Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. "Solid

phase synthesis of peptide C-terminal thioesters by Fmoc/t-Bu chemistry." *J. Am. Chem. Soc.* **1999**, *121*, 11369-11374; (3) Li, X. Q.; Kawakami, T.; Aimoto, S. "Direct preparation of peptide thioesters using an Fmoc solidphase method." *Tetrahedron Lett.* **1998**, *39*, 8669-8672; (4) Clippingdale, A. B.; Barrow, C. J.; Wade, J. D. "Peptide thioester preparation by Fmoc solid phase peptide synthesis for use in native chemical ligation." *J. Pept. Sci.* **2000**, *6*, 225-234; and (5) Bu, X. Z.; Xie, G. Y.; Law, C. W.; Guo, Z. H. "An improved deblocking agent for direct Fmoc solidphase synthesis of peptide thioesters." *Tetrahedron Lett.* **2002**, *43*, 2419-2422). In ceratin embodiments, the model thioester is a C-terminal glycine thioester, which is locally achiral and cannot be epimerized, and is therefore easy to synthesize. Though the desired GP120 thioester contains an epimerization-prone C-terminal histidine (His) residue, such thioesters have been synthesized previously and have in fact been shown to modulate favorably the rate of NCL (See, for example, Hackeng, T. M.; Griffin, J. H.; Dawson, P. E. "Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology." *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 10068-10073).

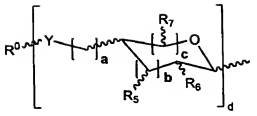
[0162] In another aspect of the present invention, a method of preparing an isolated compound having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

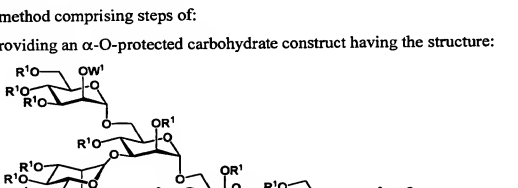


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties;

said method comprising steps of:

(a) providing an α-O-protected carbohydrate construct having the structure:



wherein R^{4A} is hydrogen or a suitable oxygen protecting group;

(b) reacting the construct of step (a) under suitable conditions to form a β-amino carbohydrate construct having the structure:

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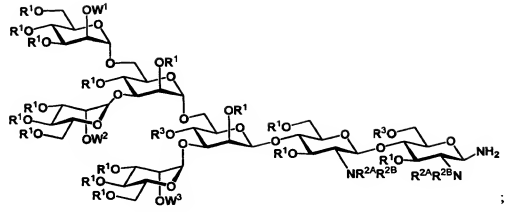
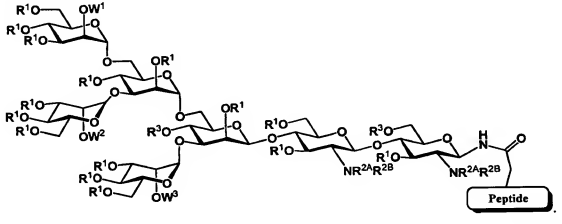
Client Reference No.: SK-1062-PRO3



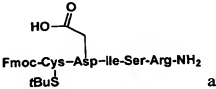
(c) reacting said β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of GP120 near an N-glycosylation site and which comprises a $-CH_2CO_2H$ moiety, to form a glycopeptide having the structure:



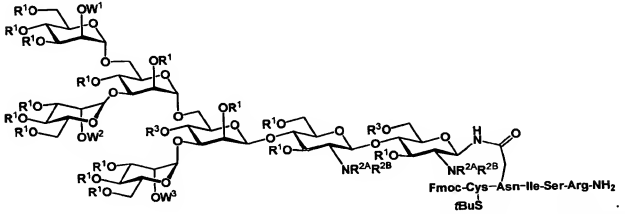
[0163] In certain exemplary embodiments, in the step of reacting the carbohydrate construct of step (a) under suitable conditions to form the β-amino carbohydrate construct, Kochetkov amination conditions are used. In certain exemplary embodiments, in the step of reacting the carbohydrate construct of step (a) under suitable conditions to form the β-amino carbohydrate construct, NH_4HCO_3/H_2O is used. In certain exemplary embodiments, in the β-amino carbohydrate construct of step (b), each occurrence of R^1 and R^3 is hydrogen and each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

[0164] In certain other exemplary embodiments, in the step of reacting the β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of GP120 near an N-glycosylation site, the reaction conditions

comprise HATU and Hünig's base in a suitable solvent. In certain embodiments, the solvent is DMSO. In certain embodiments, the peptide has the following structure:

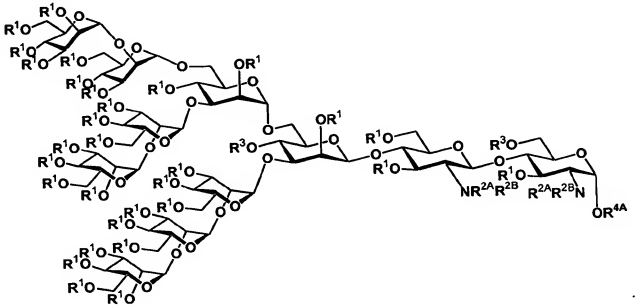


and the glycopeptide of step (c) has the structure:

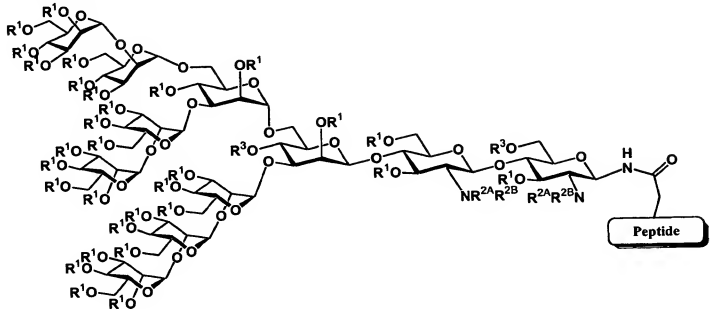


[0165] In certain exemplary embodiments, in the β-amino carbohydrate construct formed in step (b), each occurrence of R^1 and R^3 is hydrogen, each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

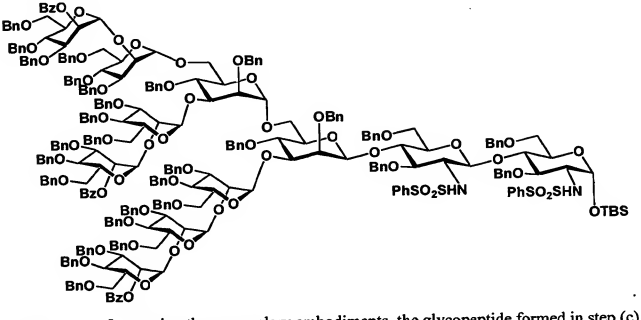
[0166] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:



[0167] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:



[0168] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:



[0169] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:

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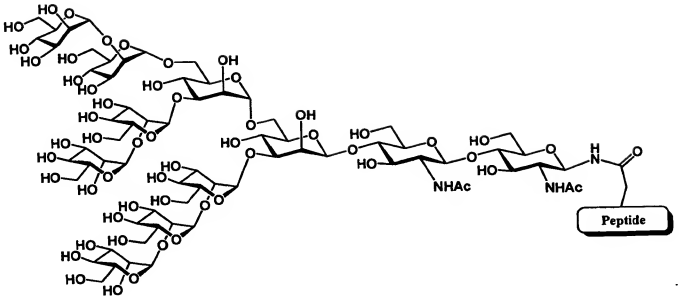
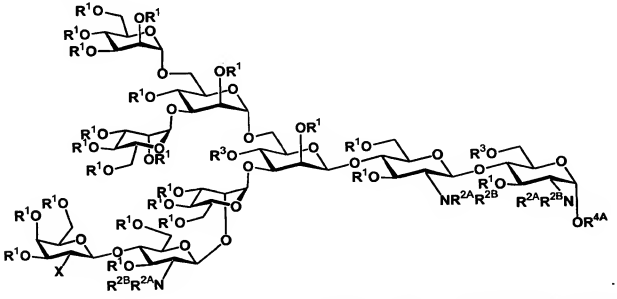
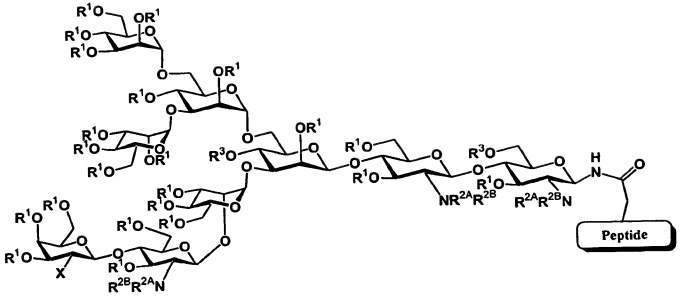
Client Reference No.: SK-1062-PRO3



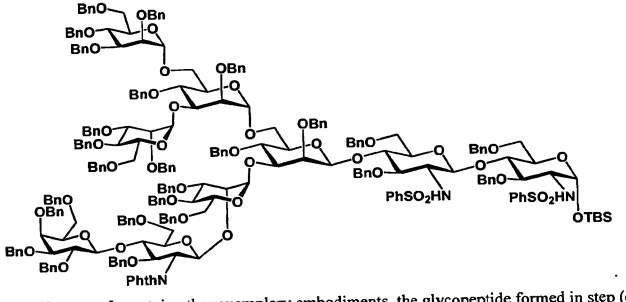
[0170] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:



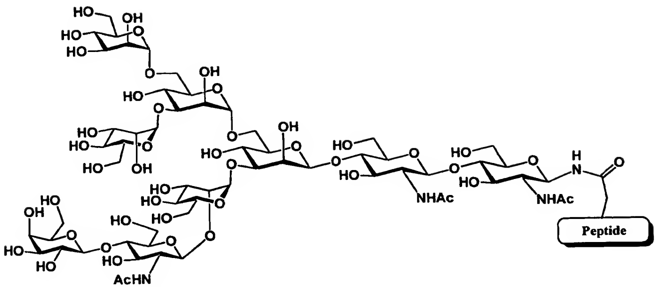
[0171] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:



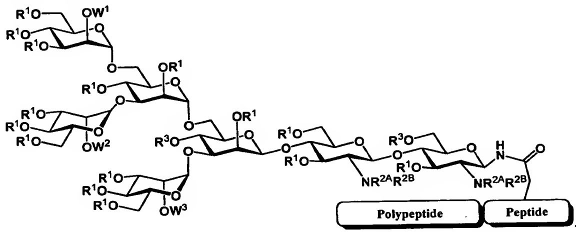
[0172] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:



[0173] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:



[0174] In certain other embodiments, the method further comprises a step of subjecting the glycopeptide formed in step (c) to Native Chemical Ligation conditions in the presence of a suitable polypeptide to form a glycopolypeptide having the structure:

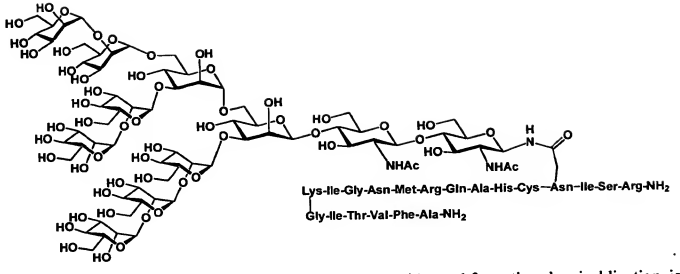


[0175] In certain embodiments, the peptide is either identical to or closely related to that of GP120 near an N-glycosylation site and comprises the amino acid sequence: Cys-Asn-Ile-Ser-Arg wherein any one or more of the amino acid residues may bear one or more protecting groups. In certain exemplary embodiments, the carbohydrate construct is attached to an Asparagine residue (Asn) on the peptide via an amide linkage. In certain other exemplary embodiments, the peptide is either identical to or closely related to that of GP120 near an N-glycosylation site and comprises the amino acid sequence:

[0176] In certain other embodiments, when the glycopeptide formed in step (c) is further subjected to Native Chemical Ligation, the polypeptide comprises the amino acid sequence: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg, wherein any one or more of the amino acid residues may bear one or more protecting groups or a moiety suitable for Native Chemical Ligation. In certain embodiments, the polypeptide comprises a moiety suitable for Native Chemical Ligation, wherein the NCL moiety comprises a thioester.

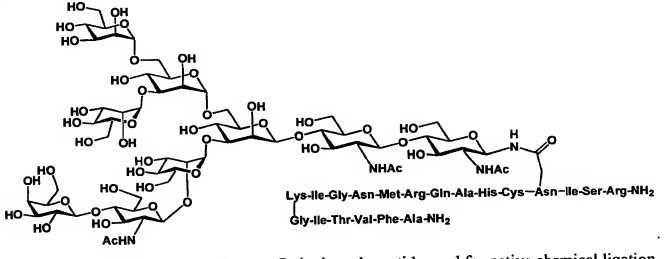
[0177] The synthetic methodology is easily applicable to the generation of significantly longer (or shorter) segments of GP120. Both the peptide to be glycosylated and the thioester utilized for NCL can more closely approach the ~60 residue limit for linear synthesis; the resulting peptide can thus extend entirely to the N-terminus of GP120. If the peptide to be glycosylated is extended significantly towards the C-terminus of GP120 the glycosylation yield might suffer due to secondary structure formation of the longer peptide (See, for example, (1) Kent, S. B. H. "Chemical Synthesis of Peptides and Proteins." *Annu. Rev. Biochem.* **1988**, *57*, 957- 989; and (2) Tam, J. P.; Lu, Y. A. "Coupling Difficulty Associated with Interchain Clustering and Phase- Transition in Solid-Phase Peptide-Synthesis." *J. Am. Chem. Soc.* **1995**, *117*, 12058-12063), but reaction conditions involving chaotropic salts have been devised to overcome issues of aggregation (See, for example, Thaler, A.; Seebach, D.; Cardinaux, F. "Lithium Salt Effects in Peptide Synthesis. 2. Improvement of Degree of Resin Swelling and of Efficiency of Coupling in Solid-Phase Synthesis." *Helv. Chim. Acta* **1991**, *74*, 628-643).

[0178] In certain exemplary embodiments, the polypeptide has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His–SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



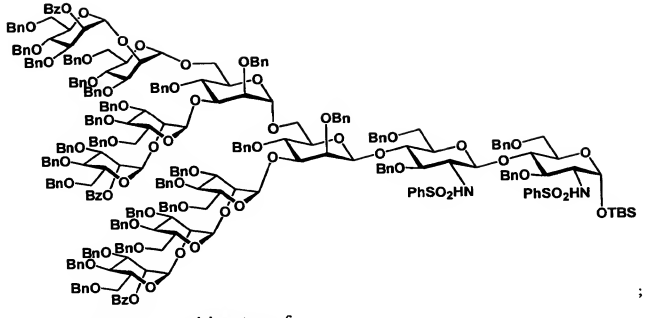
[0179] In certain embodiments, R, in the polypeptide used for native chemical ligation, is $-(CH_2)_2C(=O)NH_2$.

[0180] In certain exemplary embodiments, the polypeptide has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His–SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



[0181] In certain embodiments, R, in the polypeptide used for native chemical ligation, is $-(CH_2)_2C(=O)NH_2$.

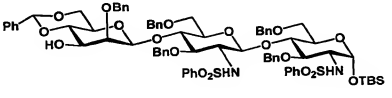
[0182] In another aspect, the invention provides a method of preparing an α-O-protected carbohydrate construct having the structure:



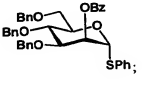
;

said method comprising steps of:

(a) coupling a trisaccharide having the structure:

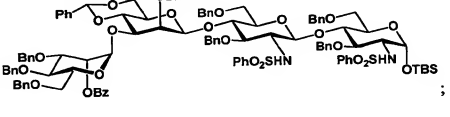


with a monosaccharide having the structure:



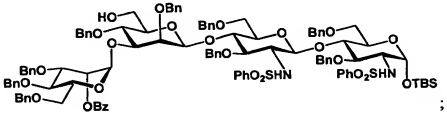
;

in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:



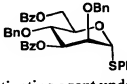
;

(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:



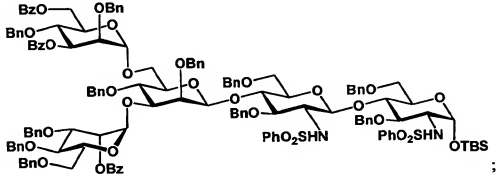
;

(c) coupling the partially deprotected tetrasaccharide formed in step (b) with a monosaccharide having the structure:



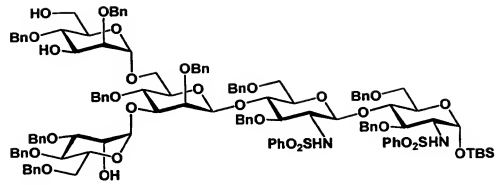
;

in the presence of an activating agent under suitable conditions to form a protected pentasaccharide having the structure:



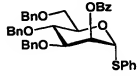
;

(d) partially deprotecting the pentasaccharide formed in step (c) under suitable conditions to form a partially deprotected pentasaccharide having the structure:

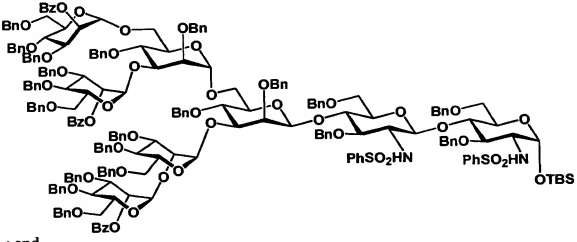


;

(e) coupling the partially deprotected pentasaccharide formed in step (d) with a monosaccharide having the structure:

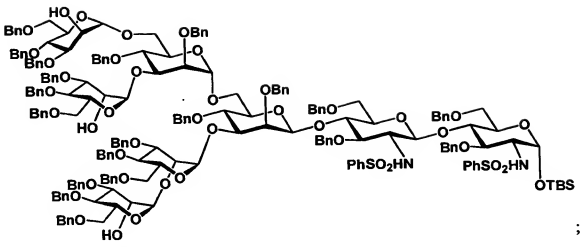


in the presence of an activating agent under suitable conditions to form an octasaccharide having the structure:



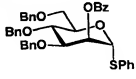
; and

(f) partially deprotecting the octasaccharide formed in step (e) under suitable conditions to form a partially deprotected octasaccharide having the structure:



; and

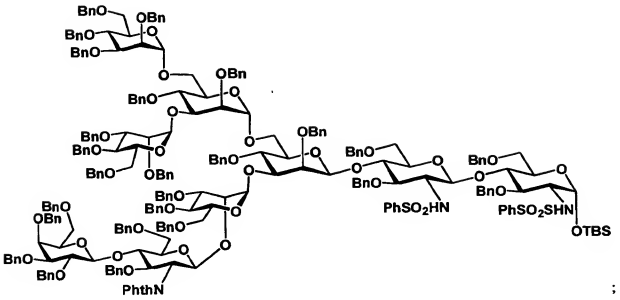
(g) coupling the partially deprotected octasaccharide formed in step (f) with a monosaccharide having the structure:



in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

[0183] In certain exemplary embodiments, the activating agent used in steps (a), (c), (e) and (g) comprises $(BrC_6H_4)_3NSbCl_6$. In certain other exemplary embodiments, in the step of partially deprotecting the protected tetrasaccharide (step (b)), the protected tetrasaccharide formed in step (a) is subjected to reductive reaction conditions comprising Bu_2BOTf, BH_3. In certain other exemplary embodiments, in the step of partially deprotecting the protected pentasaccharide (step (d)), the protected pentasaccharide formed in step (c) is subjected to reaction conditions comprising NaOMe. In certain other exemplary embodiments, in the step of partially deprotecting the protected octasaccharide (step (f)), the protected octasaccharide formed in step (e) is subjected to reaction conditions comprising NaOMe.

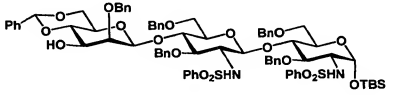
[0184] In another aspect, the invention provides a method of preparing an α-O-protected carbohydrate construct having the structure:



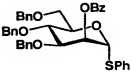
;

said method comprising steps of:

(a) coupling a trisaccharide having the structure:

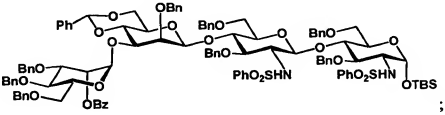


with a monosaccharide having the structure:



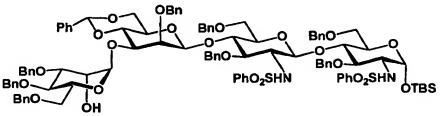
;

in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:

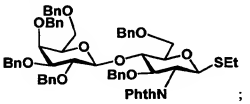


;

(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:

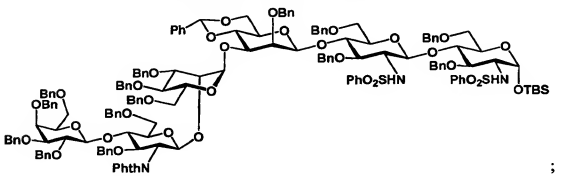


(c) coupling the partially deprotected tetrasaccharide formed in step (b) with an ethylthioglycoside having the structure:



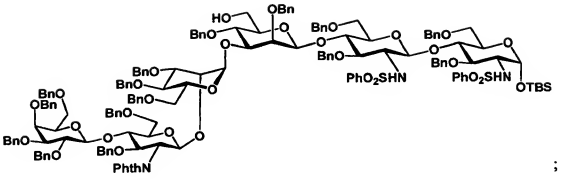
;

under suitable conditions to form a protected hexasaccharide having the structure:



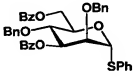
;

(d) partially deprotecting the hexasaccharide formed in step (c) under suitable conditions to form a partially deprotected hexasaccharide having the structure:

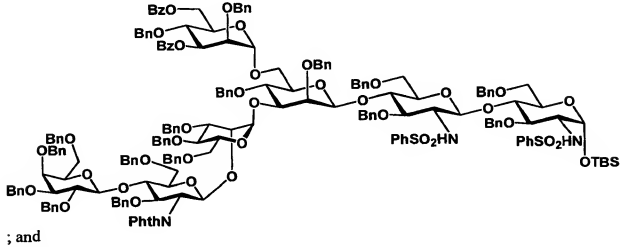


;

(e) coupling the partially deprotected hexasaccharide formed in step (d) with a monosaccharide having the structure:

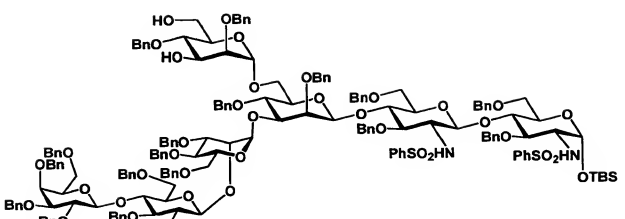


in the presence of an activating agent under suitable conditions to form an heptasaccharide having the structure:



; and

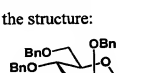
(f) partially deprotecting the heptasaccharide formed in step (e) under suitable conditions to form a partially deprotected heptasaccharide having the structure:



;

and

(g) coupling the partially deprotected heptasaccharide formed in step (f) with a monosaccharide having the structure:



in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

[0185] In certain exemplary embodiments, the activating agent used in steps (a), (e) and (g) comprises $(BrC_6H_4)_3NSbCl_6$. In certain other exemplary embodiments, in the step of partially deprotecting the protected hexasaccharide (step (d)), the protected hexasaccharide formed in step (c) is subjected to reductive reaction conditions comprising Bu_2BOTf, BH_3. In certain other exemplary embodiments, in the step of partially deprotecting the protected tetrasaccharide (step (b)), the protected tetrasaccharide formed in step (a) is subjected to reaction conditions comprising NaOMe. In certain other exemplary embodiments, in the step of partially deprotecting the protected heptasaccharide (step (f)), the protected heptasaccharide formed in step (e) is subjected to reaction conditions comprising NaOMe.

[0186] It will be appreciated that for each of the methods as detailed herein, the full arsenal of protecting groups known in the art of organic synthesis can be utilized, for example, as set forth in "Activating Agents and Protecting Groups: Handbook of Reagents for Organic Synthesis" Roush, W.R. and Pearson, A.J., Eds., John Wiley & Sons: 1999; and "Protective Groups in Organic Synthesis" Greene, T.W. and Wuts, P.G., John Wiley & Sons, New York:

1999, the entire contents of which are hereby incorporated by reference. In but a few examples, suitable protecting groups utilized herein include, but are not limited to, Bn (benzyl), TIPS (triisopropylsilyl), and Ac (acetate). In a certain exemplary embodiments of the present invention, coupling of glycoside moieties are effected under MeOTf promotion, as described herein. It will be appreciated by one of ordinary skill in the art however, that a variety of conditions known in the art of organic synthesis can be utilized to effect coupling of glycoside moieties.

[0187] The skilled practitioner will know how to adapt the synthetic methods detailed in the present invention to access a variety of other multi-branched GP120 glycans and glycopeptides thereof.

[0188] In certain other exemplary embodiments, the construct should be so functionalized as to anticipate the need for its conjugation to an immunogenic carrier (*e.g.*, protein or lipid) in anticipation of the need to stimulate an immune response. As discussed above, such constructs may be used to generate antibodies for use in HIV vaccine. The present invention provides improvements in total synthesis and HIV therapy. For example, as discussed exetensively herein, the present invention provides novel glycopeptide synthetic methodology that allows access to complex glycans *N*-linked to peptide backbones.

[0189] As discussed above, in one embodiment of the present invention, the inventive compounds can be conjugated either directly or through a crosslinker to an appropriate carrier (*e.g.*, KLH) to generate a synthetic tumor antigen. Methods of conjugation are well known in the art. For example, a conjugation strategy may be employed that involves a reductive coupling of an aldehyde (CHO) functionality on the antigenic compound, with the intended protein carrier, or lipid, presumably at the ε-amino acid residues of exposed lysines. (M.A. Bernstein; L.D. Hall, *Carbohydr. Res.* **1980**, *78*, C1; R.V. Lemieux *Chem. Soc. Rev.* **1978**, *7*, 423). Thus, in another aspect, the present invention provides synthetic constructs, whereby novel antigenic structures, as described herein, are conjugated to immunogenic carriers (*e.g.*, proteins, peptides or lipids).

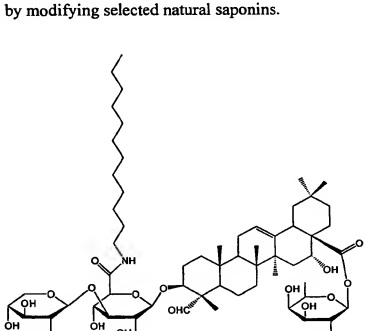
[0190] In summary, there is provided a method for GP120 glycan synthesis that is easily modified to incorporate higher degrees of carbohydrate branching. In addition, the inventive synthetic method allows the incorporation of synthetic glycans into relatively long GP120 peptides using a fast, high-yielding strategy that remains synthetically flexible. Accordingly, the

glycopeptide structures may be optimized based on their abilities to generate antibodies for use in an HIV vaccine.

[0191] **3) Compositions**

[0192] In another aspect, the present invention provides compositions comprising any one or more of the inventive GP120 glycans and/or glycopeptides.

[0193] In certain embodiments, the inventive compositions may comprise an adjuvant. In certain embodiments, the adjuvant is a saponin adjuvant (see, *e.g.*, Marciani *et al.*, *Vaccine*, **2000**, *18*, 3141, US Patent No.: 6,080,725 and 5,977,081, the entire contents of which are hereby incorporated by reference). One example of a preferred saponin adjuvant includes, but is not limited to, GPI-0100, (Galenica Pharmaceuticals, Inc., Frederick, MD) which is a semi-synthetic adjuvant derived by modifying selected natural saponins.



GPI-0100

[0194] Saponins isolated from *Quillaja soponaria Molina* contain two acyl moieties, a normonoterpene carboxylic acid and a normonoterpene carboxylic acid glycoside, which are linked linearly to a fucosyl residue attached at position C-28. It has been hypothesized that these lipophilic acyl groups may be responsible for these saponins' toxicity and their ability to stimulate cytotoxic T cells against exogenous antigens. The linkage between the fucosyl residue

and the acyl group is unstable and hydrolyzes under mild conditions (pH≥6) with concomittant loss of saponins capability to stimulate cell-mediated immune response. Unlike their saponin precursors, GPI-0100 adjuvants comprise a stable non-toxic lipophilic moiety in the saponin's glucuronic residue. Methods for preparing these semi-synthetic adjuvants are well-known in the art. For example, GPI-0100 adjuvants may be prepared by hydrolizing quillaja saponins (which are commercially available) under basic conditions to yield the corresponding deacylated product. The deacylated intermediate may then be reacted with a suitable amine reagent using standard carboxylic acid moiety activation methodology to give the desired compounds. A wide variety of procedures are effective for extrating saponin compounds. They are generalized as follows: (i) defatting of the organic matter with a hydrophobic organic solvent such as petroleum ether; (ii) extraction with a suitable alcohol (*e.g.*, methanol or ethanol) or alcohol-water mixture; (iii) evaporation of the carinol solvent; and (iv) partitioning of the dried alcohol extract between water and n-butanol saturated with water, followed by precipitation of the crude saponins from the n-butanol/water with a suitable organic solvent (*e.g.*, diethyl ether). Purification of the saponin extract may require multiple separation steps. For example, preliminary fractionation may be carried out using conventional open column chromatography or flash chromatography on silica gel, in combination with a more sophisticated chromatographic technique such as High Pressure Liquid Chromatography (HPLC), droplet counter-current liquid chromatography (DCCC) or centrifugal Liquid Chromatography (RLCC). The integration of these techniques with preparative TLC typically affords separated and purified saponins.

[0195] In certain other preferred embodiments, the adjuvant is bacteria or liposomes. In certain examples, the adjuvant includes but is not limited to, *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

[0196] As described above, the present invention provides compounds and synthetic methodologies useful in the development of novel therapeutic agents, particularly for fully synthetic HIV vaccines and/or therapeutics. In general, the compounds and glycopeptides prepared as disclosed herein can be conjugated to a protein carrier or a lipid to generate useful glycoconjugates for the treatment and/or prevention of HIV in a subject suffering therefrom. In addition, glycoconjugates prepared by processes disclosed herein are useful in adjuvant therapies as vaccines capable of inducing a potent and broad neutralizing antibody response. Such adjuvant therapies may reduce the rate of progression of HIV and/or prevent the onset of HIV.

[0197] Thus, the present invention provides pharmaceutical compositions for treating HIV, and for preventing the onset or progression of HIV, comprising any of the compounds of the present invention disclosed herein, as an active ingredient, optionally, though typically in combination with a pharmaceutically acceptable carrier. The pharmaceutical compositions of the present invention may further comprise other therapeutically active ingredients (*e.g.*, anti-HIV and/or palliative agents). For purposes of the invention, the term *"Palliative"* refers to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, palliative treatment encompasses painkillers, antinausea medications and anti-sickness drugs.

[0198] The inventive compositions include those suitable for oral, rectal, topical (including transdermal devices, aerosols, creams, ointments, lotions and dusting powders), parenteral (including subcutaneous, intramuscular, and intravenous), ocular (opthalmic), pulmonary (nasal or buccal inhalation) or nasal administration. Although the most suitable route in any given case will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[0199] In preparing oral dosage forms, any of the unusual pharmaceutical media may be used, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (*e.g.*, suspensions, elixers and solutions); or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disinterating agents, etc., in the case of oral solid preparations are preferred over liquid oral preparations such as powders, capsules and tablets. If desired, capsules may be coated by standard aqueous or non-aqueous techniques. In addition to the dosage forms described above, the compounds of the invention may be administered by controlled release means and devices.

[0200] Pharmaceutical compositions of the present invention suitable for oral administration may be prepared as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient in powder or granular form or as a solution or suspension in an aqueous or nonaqueous liquid or in an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any of the methods known in the art of pharmacy. In general, compositions are prepared by uniformly and intimately admixing the

active ingredient with liquid carriers, finely divided solid carriers, or both and then, if necessary, shaping the product into the desired form. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granule optionally mixed with a binder, lubricant, inert diluent or surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

[0201] **4) Pharmaceutical Uses and Methods of Treatment**

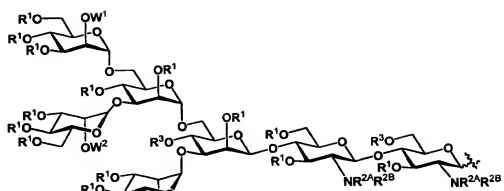
[0202] *Pharmaceutical Uses*

[0203] In one aspect, the present invention provides GP120 glycans and *N*-linked GP120 glycopeptides thereof for use in developing a pharmaceutical agent useful for preventing or reducing the rate of infection with HIV in subjects.

[0204] In another aspect, the inventive GP120 glycans and N-linked glycopeptides thereof may be used to raise antibodies specific to HIV virus. In another aspect, the invention provides an antibody which is specific to any one of the gp120 glycans and/or glycopeptides described herein, independently of the others.

[0205] Accordingly, in one aspect of the invention, there is provided an antibody or antibody fragment which to any one of the inventive GP120 antigens (independently of the others), said antibody being a purified polyclonal antibody or a monoclonal antibody. As used herein, the term "antibody fragment" is generally intended to mean any antibody fragment having conserved the specificity of the antibody of origin, and in particular fragments of the Fab and F(ab^1) type. Unless otherwise indicated, the term "antibody" also subsequently denotes antibody fragments when appropriate. The expression "antibody which binds specifically to GP120 antigen" or "antibody which is specific to GP120 antigen" is intended to denote, an antibody which binds to any one GP120 glycans and N-linked glycopeptides thereof (independently of the others) described herein with high specificity. For example, in certain embodiments, the product which is bound to the antibody consists of at least 80% and preferably of at least 90%, of said GP120 antigen.

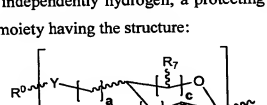
[0206] Thus, in one aspect, the invention provides an antibody or antibody fragment which is specific to any one of the inventive antigens (independently of the others) containing a carbohydrate domain having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

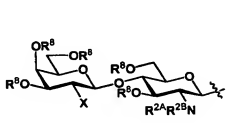


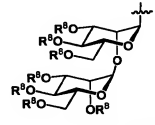
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties;

and wherein said antibody is a purified polyclonal antibody or a monoclonal antibody. In certain embodiments, the antibody is a monoclonal antibody.

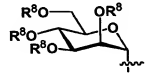
[0201] In certain embodiments, W^3 is R^1, R^3, as defined above, or a moiety having the structure:



or


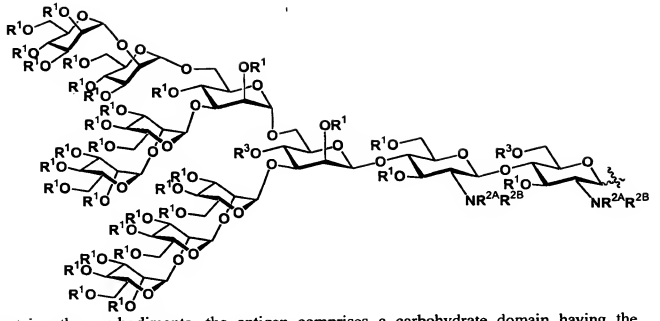
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

[0202] In certain embodiments, W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:

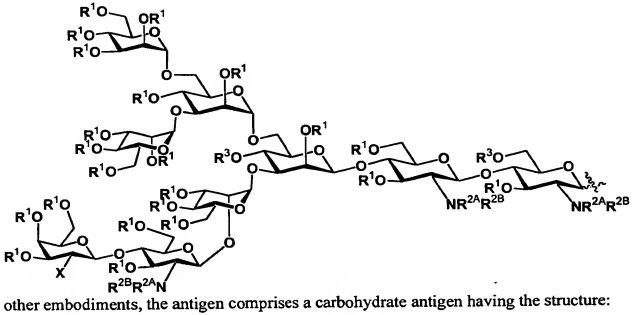


wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

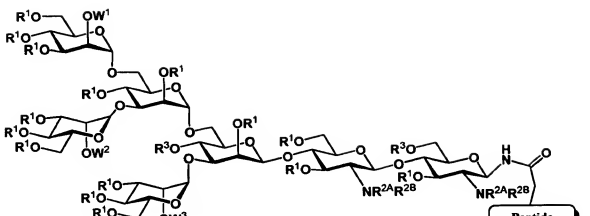
[0203] In certain other embodiments, the antigen comprises a carbohydrate domain having the structure:



. In certain other embodiments, the antigen comprises a carbohydrate domain having the structure:



In yet other embodiments, the antigen comprises a carbohydrate antigen having the structure:

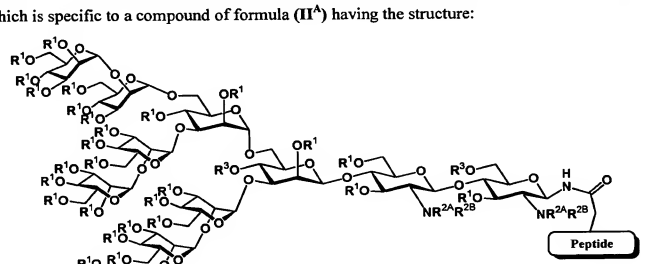


;

(I^A)

wherein the peptide has a structure either identical to or closely related to that of GP120 near an N-glycosylation site.

[0204] In certain embodiments, the invention provides an antibody or antibody fragment which is specific to a compound of formula **(II^A)** having the structure:



;

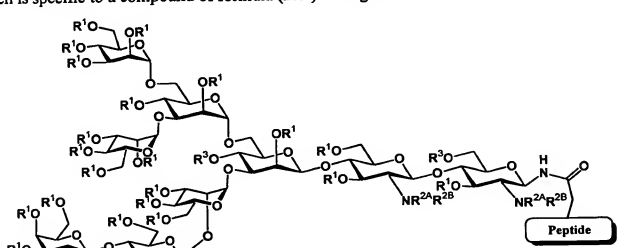
(II^A)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group; each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group; and each occurrence of R^3 is independently hydrogen or a protecting group;

wherein the peptide has a structure either identical to or closely related to that of GP120 near an N-glycosylation site;

and wherein said antibody is a purified polyclonal antibody or a monoclonal antibody.

[0205] In certain embodiments, the inIvention provides an antibody or antibody fragment which is specific to a compound of formula (III^A) having the structure:



;

(III^A)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group; each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group; and each occurrence of R^3 is independently hydrogen or a protecting group;

wherein the peptide has a structure either identical to or closely related to that of GP120 near an N-glycosylation site;

and wherein said antibody is a purified polyclonal antibody or a monoclonal antibody.

[0206] In certain exemplary embodiments, the antibody is a monoclonal antibody.

[0207] The glycopeptides of the invention may be used to prepare monoclonal or polyclonal antibodies. Conventional methods can be used to prepare the antibodies. As to the details relating to the preparation of monoclonal antibodies reference can be made to Goding, J. W., Monoclonal Antibodies: Principles and Practice, 2nd Ed., Academic Press, London, 1986.

[0208] The glycopeptides and antibodies specific for the GP120 glycans and/or glycopeptides of the invention may be labelled using conventional methods with various enzymes, fluorescent materials, luminescent materials and radioactive material. Linking an antibody or an antibody fragment to a label, whether it is a radioactive, enzymatic or colored label or any other type of label commonly used in immunological techniques, is well known and described in the literature. Suitable enzymes, fluorescent materials, luminescent materials, and radioactive material are well known to the skilled artisan.

[0209] It is presently unknown, however, how large a segment of GP120 is required to generate appropriate antibodies; e.g., the glycopeptide may not have enough native structure to develop appropriately specific antibodies. The glycopeptide might not itself be immunogenic, and could therefore require the use of an adjuvant to stimulate an immune response. Examples of suitable adjuvants include, but are not limited to, saponin adjuvants (*e.g.*, GPI-0100), *Salmonella minnesota* cells, bacille Calmette-Guerin and/or QS21.

[0210] A lack of immune response with any length glycopeptide would call for the use of a carrier protein such as keyhole limpet hemocyanin (KLH), [34-36] an adjuvant [37] such as covalently bound Pam_3Cys, [38] or coadministered QS21. [39] Such immunostimulants have been used alone or in concert[40-42] to generate antibodies from small glycopeptide haptens, [43-45] and should prove effective here, as well. Though the first two systems require covalent conjugation, the synthetic design allows late-stage conjugation as demonstrated previously for other glycopeptides.[46]

[0211] **References**

34. Ragupathi, G. "Carbohydrate antigens as targets for active specific immunotherapy." *Cancer Immunol. Immun.* **1996**, *43*, 152-157.

35. Helling, F.; Shang, A.; Calves, M.; Zhang, S. L.; Ren, S. L.; Yu, R. K.; Oettgen, H. F.; Livingston, P. O. "G(D3) Vaccines for Melanoma - Superior Immunogenicity of Keyhole Limpet Hemocyanin Conjugate Vaccines." *Cancer Res.* **1994**, *54*, 197-203.

36. Harris, J. R.; Markl, J. "Keyhole limpet hemocyanin (KLH): A biomedical review." *Micron* **1999**, *30*, 597-623.

37. Kellner, J.; Erhard, M.; Schranner, I.; Losch, U. "The Influence of Various Adjuvants on Antibody Synthesis Following Immunization with an Hapten." *Biol. Chem. Hoppe-Seyler* **1992**, *373*, 51-55.

38. Metzger, J.; Wiesmuller, K. H.; Schaude, R.; Bessler, W. G.; Jung, G. "Synthesis of Novel Immunologically Active Tripalmitoyl-S- Glycerylcysteinyl Lipopeptides as Useful Intermediates for Immunogen Preparations." *Int. J. Pept. Protein Res.* **1991**, *37*, 46-57.

39. Kensil, C. R.; Patel, U.; Lennick, M.; Marciani, D. "Separation and Characterization of Saponins with Adjuvant Activity from Quillaja-Saponaria Molina Cortex." *J. Immunol.* **1991**, *146*, 431-437.

40. Livingston, P. O.; Adluri, S.; Helling, F.; Yao, T. J.; Kensil, C. R.; Newman, M. J.; Marciani, D. "Phase-1 Trial of Immunological Adjuvant QS-21 with a GM2 Ganglioside-Keyhole Limpet Hemocyanin Conjugate Vaccine in Patients with Malignant-Melanoma." *Vaccine* **1994**, *12*, 1275-1280.

41. Zhang, S. L.; Graeber, L. A.; Helling, F.; Ragupathi, G.; Adluri, S.; Lloyd, K. O.; Livingston, P. O. "Augmenting the immunogenicity of synthetic MUC1 peptide vaccines in mice." *Cancer Res.* **1996**, *56*, 3315-3319.

42. Musselli, C.; Livingston, P. O.; Ragupathi, G. "Keyhole limpet hemocyanin conjugate vaccines against cancer: the Memorial Sloan Kettering experience." *J. Cancer Res. Clin. Oncol.* **2001**, *127*, R20- R26.

43. Adluri, S.; Helling, F.; Ogata, S.; Zhang, S. L.; Itzkowitz, S. H.; Lloyd, K. O.; Livingston, P. O. "Immunogenicity of Synthetic TF-KLH (Keyhole Limpet Hemocyanin) and STn-KLH Conjugates in Colorectal Carcinoma Patients." *Cancer Immunol. Immun.* **1995**, *41*, 185-192.

44. Kudryashov, V.; Glunz, P. W.; Williams, L. J.; Hintermann, S.; Danishefsky, S. J.; Lloyd, K. O. "Toward optimized carbohydrate-based anticancer vaccines: Epitope clustering, carrier structure, and adjuvant all influence antibody responses to Lewis(y) conjugates in mice." *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3264-3269.

45. Ragupathi, G.; Howard, L.; Cappello, S.; Koganty, R. R.; Qiu, D. X.; Longenecker, B. M.; Reddish, M. A.; Lloyd, K. O.; Livingston, P. O. "Vaccines prepared with sialyl-Tn and sialyl-Tn trimers using the 4-(4-maleimidomethyl)cyclohexane-1-carboxyl hydrazide linker group result in optimal antibody titers against ovine submaxillary mucin and sialyl-Tn-positive tumor cells." *Cancer Immunol. Immun.* **1999**, *48*, 1-8.

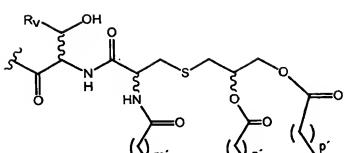
46. Glunz, P. W.; Hintermann, S.; Williams, L. J.; Schwarz, J. B.; Kuduk, S. D.; Kudryashov, V.; Lloyd, K. O.; Danishefsky, S. J. "Design and synthesis of Le(y)-bearing glycopeptides that mimic cell surface Le(y) mucin glycoprotein architecture." *J. Am. Chem. Soc.* **2000**, *122*, 7273-7279.

[0212] *Methods of Treatment*

[0213] As detailed above, a major drawback in using carbohydrate epitopes, is that they are generally not readily available by isolation from natural sources. For example, the immense difficulties associated with their purification from natural sources render them virtually

nonavailable as homogeneous starting materials for a clinical program. Thus, the incorporation of these naturally occurring epitopes into carrier proteins/peptides or any favorable molecular context via conjugation for eliciting a therapeutically useful immunological response is inefficient at best, and often virtually impossible. Therefore, to effectively study vaccines as therapeutic agents, sufficient material can only be obtained by chemical synthesis. As discussed above, the present invention provides a variety of synthetic glycoforms of GP120 (carbohydrate constructs and glycopeptide conjugates), and methods for preparing them.

[0214] Accordingly, in another aspect of the invention, a method of treatment is provided comprising administering to a subject in need thereof a therapeutically effective amount of any of the GP120 glycans and/or glyconjugates thereof disclosed herein (*e.g.*, glycopeptides, which may additionally be conjugated to a protein, peptide or lipid carrier, either directly or through a crosslinker), optionally in combination with a pharmaceutically acceptable carrier. In certain embodiments, a method for preventing the infection with HIV is provided comprising administering to a subject in need thereof a therapeutically effective amount of any of the GP120 glycans and/or glyconjugates thereof disclosed herein, optionally in combination with an adjuvant. In certain embodiments, a method for the treatment of HIV is provided comprising administering to a subject in need thereof a therapeutically effective amount of any of the GP120 glycans and/or glyconjugates thereof disclosed herein, optionally in combination with an adjuvant. In certain embodiments, a method for inducing antibodies in a human subject, wherein the antibodies are specific to a carbohydrate antigen expressed on the surface of gp120, which comprises administering to the subject an amount of any of the glycans and/or glycoconjugates disclosed above effective to induce antibodies. In certain embodiments, the method utilized any one or more of the GP120 glycans and/or glycopeptides thereof disclosed herein, where the glycan(s) and/or glycopeptide(s) is/are linked to an immunogenic carrier either directly or through a crosslinker, which carrier is a protein, peptide or lipid. In certain embodiments, the carrier is Bovine Serum Albumin, polylysine or KLH. In certain other embodiments, the carrier is a lipid having the structure:



wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys).

[0215] In certain other embodiments, the method comprises administering to a subject in need thereof a therapeutically effective amount of any of the compounds and/or glycopeptides disclosed herein, in combination with an immunogenic carrier, optionally in combination with a pharmaceutically acceptable carrier. Specifically, in certain exemplary embodiments, the method comprises administering a GP120 glycan and/or glycopeptide additionally conjugated to an immunogenic carrier. In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of any one or more of the glyconjugates disclosed herein (*e.g.*, glycopeptides, which may additionally be conjugated to a protein, peptide or lipid carrier, either directly or through a crosslinker), in combination with an immunogenic carrier, optionally in combination with a pharmaceutically acceptable carrier. In certain embodiments, the method comprises administering one or more GP120 glycans and/or glycopeptides and an immunogenic carrier that have not been conjugated. Rather, they are administered concurrently, or successively, as separate entities. In certain other exemplary embodiments, the method comprises administering one or more GP120 glycan and/or glycopeptide of the invention conjugated (*i.e.*, covalently linked) to an immunogenic carrier. In certain embodiments, the method comprises administering any one or more inventive GP120 glycans and/or glycopeptides disclosed herein that have not been conjugated to an immunogenic carrier. Rather, the GP120 glycan(s) and/or glycopeptide(s) and the immunogenic carrier are administered concurrently, or successively, as separate entities. In certain embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain exemplary embodiments, the carrier is Bovine Serum Albumin, polylysine or KLH. In certain other embodiments, the carrier is PamCys. For the purpose of the

invention, a compound/glycopeptide and a carrier are said to be administrered concurrently when they are administered (i) as a single composition containing the compound/glycopeptide and the carrier, (ii) as two separate compositions or (iii) are delivered by separate routes within a short enough period of time that the effective result is equivalent to that obatined when both compound/glycopeptide and carrier are administered as a single composition.

[0216] In still other embodiments, the present invention provides the related method of inducing antibodies which further comprises co-administering an immunological adjuvant, or a combination of immunological adjuvants.

[0217] In certain exemplary embodiments, the inventive GP120 glycans and glycopeptides thereof comprise carbohydrate domains, or truncated or elongated versions thereof, that are found on the surface of gp120. In certain exemplary embodiments, the inventive glycopeptides comprise peptidic domains, or truncated or elongated versions thereof, that are found near an N-glycosylation site of naturally occurring GP120.

[0218] Accordingly, embodiments of this invention encompass methods of eliciting immune responses in animals comprising administering effective amounts of inventive GP120 glycans and/or glycopeptide(s) thereof and/or compositions of the invention wherein the immune response is directed against on eor more carbohydrates expressed on the surface of gp120.

[0219] A further embodiment of this invention encompasses a use of effective amounts of inventive GP120 glycans and/or glycopeptide(s) thereof and/or a composition of the present invention to elicit an immune response in an animal preferably to treat and/or prevent HIV. The present invention further includes a use of effective amounts of inventive GP120 glycans and/or glycopeptide(s) thereof and/or a composition of the present invention to prepare a medicament to elicit an immune response in animal, preferably to treat and/or prevent HIV.

[0220] It will be appreciated that the magnitude of the therapeutic dose of the compounds of the invention will vary with the nature and severity of the condition to be treated and with the particular compound and its route of administration. In general, the daily dose range for antiHIV activity lies in the range of 0.0001 to 1.0 mg/kg of body weight in a mammal, although the present invention is not intended to be limited by this range.

[0221] Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a compound disclosed herein. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, etc. routes may be employed. Dosage forms

include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, etc. In preferred embodiments, the effective dosage is employed using a syringe injection.

[0222] It will be appreciated by one of ordinary skill in the art, however, that the most suitable route for administration will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. As discussed above, the inventive therapeutics may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[0223] Additionally, once a synthetic vaccine has been derivatized and characterized, mouse immunological studies can be performed to assess the potency and/or specificity of the novel HIV vaccines.

KITS OF THE INVENTION

[0224] In other embodiments, the present invention relates to a kit for conveniently and effectively carrying out the methods in accordance with the present invention. In general, the pharmaceutical pack or kit comprises one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Such kits are especially suited for the delivery of solid oral forms such as tablets or capsules. Such a kit preferably includes a number of unit dosages, and may also include a card having the dosages oriented in the order of their intended use. If desired, a memory aid can be provided, for example in the form of numbers, letters, or other markings or with a calendar insert, designating the days in the treatment schedule in which the dosages can be administered. Alternatively, placebo dosages, or calcium dietary supplements, either in a form similar to or distinct from the dosages of the pharmaceutical compositions, can be included to provide a kit in which a dosage is taken every day. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EQUIVALENTS

[0225] The representative examples which follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the

invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. In but one illustrative example, protecting groups play an important role in the synthesis of the carbohydrate domains and synthetic conjugates, as described herein; however it will be appreciated by one of ordinary skill in the art that the present invention encompasses the use of various alternate protecting groups known in the art. Those protecting groups used in the disclosure including the Examples below are merely illustrative.

[0226] It should further be appreciated that, uless otherwise indicated, the contents of those cited references are incorporated herein by reference to help illustrate the state of the art. The following examples contain important additional information, exemplification and guidance which can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

EXEMPLIFICATION

Example 1: Novel Method for Native Chemical Ligation:

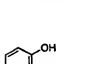
Reagents: All commercial materials were used as received unless otherwise noted. The following solvents were obtained from a dry solvent system and used without further purification: THF, diethyl ether, toluene, and DCM. Reagents were obtained from Aldrich or as noted, with the following exceptions: amino acids and resins for solid phase peptide synthesis were purchased from NovaBiochem; Biosynthesis grade DMF from EM Science; and all other solvents from Fisher Scientific (HPLC grade).

HPLC: All separations involved a mobile phase of 0.05% TFA (v/v) in water (solvent A)/0.0425% TFA in acetonitrile (solvent B). Preparative, semipreparative, and analytical HPLC separations were performed using a Rainin HXPL solvent delivery system equipped with a Rainin UV-1 detector and one of the following Dynamax-60Å C18 axial compression columns 250 mm in length equipped with a similarly packed guard column: 41.4 mm diameter (prep), 21.4 m diameter (semiprep), or 4.6 mm diameter (analytical). Separations were performed at flow rates of 48 mL/min (prep), 16 mL/min (semiprep), or 1 mL/min (analytical), and were

monitored at a wavelength between 214 and 230 nm, depending on column loading. LCMS chromatographic separations were performed using a Waters 2695 Separations Module and a Waters 996 Photodiode Array Detector equipped with a Varian Microsorb C18 2 x 150 mm column at a flow rate of 0.2 mL/min.

ESMS and LCMS: Electrospray mass spectroscopy and LCMS analyses were obtained on a Waters Micromass ZQ mass spectrometer in conjunction with the Waters HPLC apparatus described above.

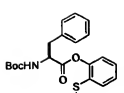
NMR: ^1H and ^{13}C NMR spectra were recorded on Bruker instruments in $CDCl_3$, CD_3OD or D_2O at 400 or 500 MHz for ^1H and 100 or 125 MHz for ^{13}C.



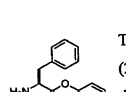
To a stirred, biphasic solution of 2-mercaptophenol (1.0 g, 7.9 mmol) in H_2O (5.1 mL) was added, drop wise, a solution of iodine (1.0 g 4.0 mmol) in methanol (3.5 mL). When the brown iodine color persisted the solution was diluted with ethyl acetate and water. The aqueous layer was removed and extracted with an additional portion of ethyl acetate. The combined organic layers were dried and washed with brine then dried (Na_2SO_4) and concentrated to give a brown oil which was used without purification (1.5 g). The product still contains iodine. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.33-7.37 (m, 2H), 7.22-7.24 (m, 2H), 6.99-7.01 (m, 2H), 6.82-6.85 (m, 2H), 6.22 (brs, 2H). ^{13}C ($CDCl_3$, 125 MHz): δ 157.3, 136.6, 133.6, 121.4, 120.3, 116.1. ESI-MS: Calcd. for $C_{12}H_{10}O_2S_2$ $[M+NH_4]^+$ 267.8 Found: 267.8.

To a stirred solution of the disulfide (1.5 g crude, ~4 mmol) in CH_2Cl_2 (25 mL) was added ethyldisulfide (10.5 mL, 79.9 mmol) and then BF_3•OEt_2 (10.1 mL, 79.9 mmol). The reaction was stirred at room temperature for three hours and then carefully quenched by the addition of $NaHCO_3$. The organic layer was drained and the aqueous layer was extracted with an additional portion of CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated to give a yellow oil. Purification by silica gel chromatography (20% ethyl acetate in hexane) gave the desired product as a clear, slightly yellow oil (1.45 g, 99%).^1H

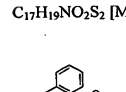
NMR ($CDCl_3$, 500 MHz): δ 7.48-7.51 (m, 1H), 7.28-7.32 (m, 1H), 6.99-7.01 (m, 1H), 6.86-6.9 (m, 1H), 6.34 (brs, 1H), 2.78 (q, J = 7.4 Hz, 2H), 1.35 (t, J = 7.4 Hz, 3 H). ^{13}C ($CDCl_3$, 125 MHz): δ 156.9, 135.2, 132.2, 121.0, 116.2, 32.4, 14.1. ESI-MS: Calcd. for $C_8H_{10}OS_2$ $[M+Na]^+$ 208.8 Found: 208.8.



To a well stirred solution of the phenol (1.45 g, 8 mmol) and Boc-Phe-OH (2.65 g, 10 mmol) in CH_2Cl_2 (25 mL) and THF (5 mL) was added EDCI (1.92 g, 10 mmol) and DMAP (98 mg, 0.8 mmol). The resulting solution was stirred are room temperature for 18 hr at which point the volatile materials were removed *in vacuo*. The resulting oil was taken up in EtOAc and washed with 1N HCl, H_2O, and then brine. The organic layer was dried (Na_2SO_4) and concentrated to give a slightly yellow oil. Purification by silical gel chromatography (30% ethyl acetate in hexane) gave a clear, colorless oil (3.5 g, >99%). The product contains a small amount of ethyl acetate, even after one day under high vacuum. ESI-MS: Calcd. for $C_{22}H_{27}NO_4S_2$ $[M+Na]^+$ 456.0 Found: 456.0.

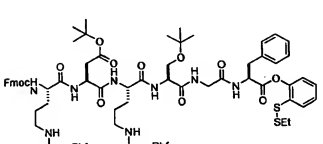


To a 50 mL polypropylene conical tube was added H_2O (5.4 mL), triethylsilane (2.6 mL, 16 mmol), and TFA (30 mL). The contents were thoroughly mixed and then added directly to the phenylalanine derivative (3.5 g, 8 mmol). The mixture was stirred for 15 min and then concentrated. H_2O was added and the mixture was again concentrated. This was repeated two more times at which point a white solid appeared at the end of concentration and the flask no longer smelled of TFA. The material was placed under high vacuum for 18 hours and then dissolved with a 30 % solution of acetonitrile in H_2O. The liquid was shell frozen and lyophilized to give a white powder. ESI-MS: Calcd. for $C_{17}H_{19}NO_2S_2$ $[M+H]^+$ 334.1 Found: 334.2.

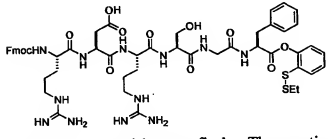


The phenylalanine derivative (15 mg, 45 μmol) and L-cysteine (6 mg, 49 μmol) were placed into a LCMS vial along with a flea-sized stirbar. In a second vial were mixed MESNa (25 mg, 150 μmol) and phosphate buffered saline (0.2M NaCl, 0.2M phosphate, pH=7.5, 2 mL). The MESNa solution was then added directly to the amino acids, and the reaction was monitored by LCMS. After two hours the reaction appeared to be complete and TCEP (129 mg, 450 μmol) was added. This was stirred for 1 hour and then injected directly onto the HPLC for purification. The desired compound was obtained as a white powder after lyophilization. ^1H NMR (D_2O): δ 7.36-7.45 (m, 3H), 7.31-7.33 (m, 2H), 4.63 (dd, J = 6.4, 5.0 Hz, 1H), 4.34 (dd, J = 7.2, 7.1 Hz, 1H), 3.29 (dd, J = 14.1, 7.1 Hz, 1H), 3.23 (dd, J =

14.1, 7.2 Hz, 1H), 2.99 (dd, J = 14.2, 5.0 Hz, 1H), 2.93 (dd, J = 14.2, 6.4 Hz, 1H). ESI-MS: Calcd. for $C_{12}H_{16}N_2O_3S$ $[M+H]^+$ 269.1, Found: 269.1



The peptide (127 mg, 0.089 mmol) and phenylalanine derivative (38 mg, 0.116 mmol) were dissolved in THF (0.16 mL) along with DMAP (1 mg, 0.009 mmol). CH_2Cl_2 (0.63 mL) was added and the mixture was stirred for 5 min. Solid EDCI (21 mg, 0.111 mmol) was added and the reaction was stirred at room temperature for 18 hr then quenched by the addition of 1N HCl and diluted with EtOAc. The aqueous layer was removed and extracted with a second portion of EtOAc. The combined organic layers were washed with water then brine, dired (Na_2SO_4) and concentrated to give a white powder. The material was purified by filtration through a small plug of silica gel (30% to 100% ethyl acetate in hexane) to give white solid (95 mg, 61%). ESI-MS: Calcd. for $C_{87}H_{114}N_{12}O_{18}S_4$ $[M+H]^+$ 1743.7, Found: 1743.7.



To a 50 mL polypropylene conical tube was added H_2O (0.7 mL), triethylsilane (0.3 mL, 1.9 mmol), phenol (678 mg, 0.082 mmol) and TFA (18 mL, 234 mmol). The contents were thoroughly mixed and then added directly to the peptide in a round bottom flask. The reaction was stirred at room temperature for three hours and then all volatile materials were removed *in vacuo*. Purification by direct injection on the HPLC (40-60%B over 20 min, rt = 15.4 min) to gave the desired material as a white powder after lyophilization (21 mg). ESI-MS: Calcd. for $C_{53}H_{66}N_{12}O_{12}S_2$ $[M+H]^+$ 1127.4, Found: 1127.4.

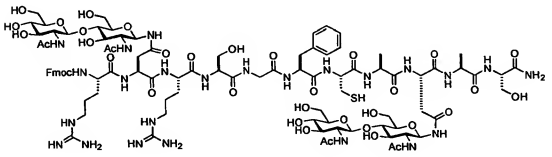
Typical aspartylation conditions:

Peptide (0.021 mmol) and HATU (0.042 mmol) were combined in a 50 ml conical tube and DMSO (0.17 mL) was added. After 5 min the resulting solution was added to a solution of the glycosylamine (0.23 mmol) in DMSO (0.17 mL). The solution was stirred for 10 min and then

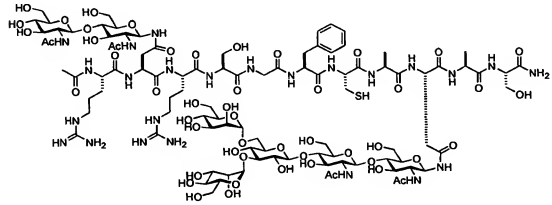
DIEA (3.7 μL, 0.21 mmol) was added. The reaction was monitored by LCMS and upon completion was injected directly on the HPLC for purification.

Typical ligation conditions:

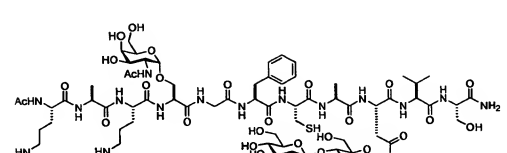
The two glycopeptide halves were placed in a LCMS vial along with a flea-sized stirbar. A stock solution of MESNa (18.3 mg, 111 mmol) in phosphate buffered saline (0.2M NaCl, 0.2M phosphate, pH=7.4, 1 mL) was made and of this, 600 μL was added to the glycopeptides. The reaction was monitored by LCMS and, once finished, TCEP (25 mg, 0.087 mmol) was added and the solution stirred for 2 hr then injected directly onto the HPLC.



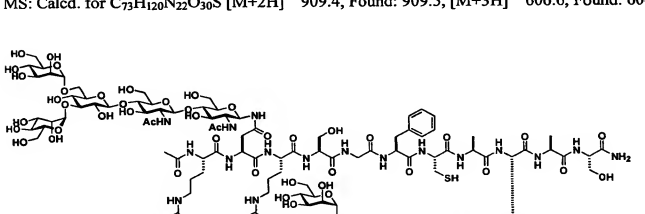
LCMS: 5-65%B over 20 min, rt = 15.17 min. HPLC: 25-55%B over 30 min, rt = 7.65 min. ESI-MS: Calcd. for $C_{93}H_{138}N_{24}O_{37}S$ $[M+2H]^{2+}$ 1108.5, Found: 1108.6, $[M+3H]^{3+}$ 739.3, Found: 739.5.



LCMS: 5-65%B over 20 min, rt = 9.56 min. HPLC: 5-65%B over 20 min, rt = 8.39 min. ESI-MS: Calcd. for $C_{98}H_{160}N_{24}O_{51}S$ $[M+2H]^{2+}$ 1261.5, Found: 1261.5, $[M+3H]^{3+}$ 841.4, Found: 841.5.



LCMS: 5-65%B over 20 min, rt = 11.40 min. HPLC: 5-65%B over 20 min, rt = 9.85 min. ESI-MS: Calcd. for $C_{73}H_{120}N_{22}O_{30}S$ $[M+2H]^{2+}$ 909.4, Found: 909.5, $[M+3H]^{3+}$ 606.6, Found: 606.8.

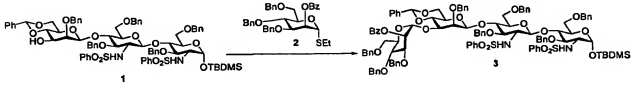


LCMS: 5-45%B over 20 min, rt = 10.36 min. HPLC: 5-45%B over 20 min, rt = 12.34 min. ESI-MS: Calcd. for $C_{116}H_{190}N_{24}O_{66}S$ $[M+2H]^{2+}$ 1504.6, Found: 1504.6. $[M+3H]^{3+}$ 1003.4, Found: 1003.7.

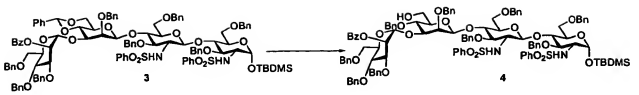
Example 2: Gp120 glycans and glycopeptides

General Methods: Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. THF, toluene, and methylene chloride was obtained from a

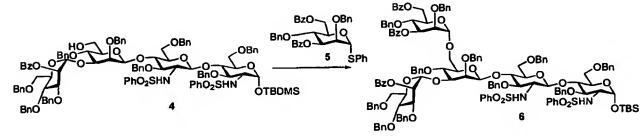
dry solvent system (passed through a prepacked column of alumina) and used without further drying. All air and water sensitive reactions were performed in flame-dried glassware under a positive pressure of prepurified argon gas. NMR (^{1}H and ^{13}C) spectra were recorded on Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz as noted individually, referenced to $CDCl_3$ (7.27 ppm for ^{1}H and 77.0 ppm for ^{13}C) or CD_3COCD_3 (2.09 ppm for ^{1}H and 30.6 and 205.9 ppm for ^{13}C). Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates. Compounds which were not UV active were visualized by dipping the plates in para-anisaldehyde solution and heating. Preparative thin layer chromatography was performed using the indicated solvent on Whatman® (LK6F Silica gel 60 Å 250 μM or Pk6F Silica Gel 60 Å 1000 μM) TLC plate.



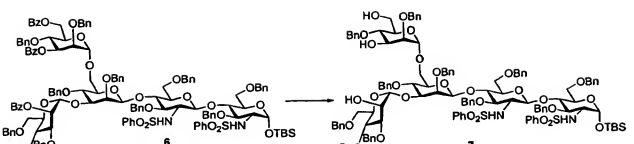
Tetrasaccharide 3: A mixture of trisacchride **1**[1] (106 mg, 0.074 mmol), thiomannoside **2** (133 mg, 0.222 mmol) and molecular sieves in CH_3CN (2 mL) was stirred for 2 h at r.t. and tris(4-bromophenyl)aminium hexachloroantimonate (199 mg, 0.244 mmol) was added at 15 °C. The solution was stirred for 4 h at r.t. and then quenched by triethylamine. The mixture was filtered through celite, concentrated, dissolved in EtOAc, filtered through silica gel and concentrated. The residue was purified by preparative TLC (PTLC) using pentane/ether (1/2) as the eluent to afford **3** as a white solid (113 mg, 78%). $[\alpha]_D^{25}$ -205.0 (*c* 0.14, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.06 (s, 3 H), 0.87 (s, 9 H), 5.07 (s, 1 H), 5.30 (s, 1 H), 5.36 (s, 1 H), 5.74 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -4.6, 13.9, 17.8, 20.8, 25.6, 57.8, 58.6, 60.1, 66.8, 67.5, 67.8, 68.2, 68.4, 68.9, 69.5, 71.0, 72.3, 73.1, 73.3, 73.5, 73.9, 74.3, 74.9, 75.1, 75.3, 75.8, 77.3, 77.6, 77.9, 78.2, 79.9, 92.6, 98.4, 100.7, 100.9, 125.6, 126.7, (126.8-129.5), 129.6, 136.9, 137.4, 137.6, 138.0, 138.1, 138.2, 138.3, 140.4, 141.3, 165.1. LRMS (ESI) calcd for $C_{112}H_{122}N_2O_{24}S_2SiNa^+$ $[M+Na]^+$ 1994.76, found 1994.8.



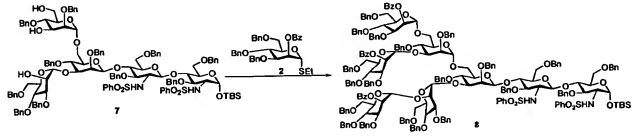
Tetrasaccharide 4: To a solution of **3** (200 mg, 0.101 mmol) in borane tetrahydrofuran etherate (1.1 mL, 1.0 M in THF, 1.01 mmol) was added dibutylboron triflate (0.334 mL, 1.0 M in CH_2Cl_2, 0.333 mmol) at 0 °C. The reaction mixture was stirred for 7 h at 0 °C and quenched with triethylamine and methanol and concentrated. The residue was purified by PTLC using pentane/ether (1/2) as the eluent to afford **4** as a white solid (172 mg, 90%). $[\alpha]_D^{25}$ -187.0 (*c* 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.08 (s, 3 H), -0.04 (s, 3 H), 0.80 (s, 9 H), 4.96 (d, *J* = 2.6 Hz, 1 H), 5.15 (s, 1 H), 5.55 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.6, 17.9, 25.7, 57.9, 58.3, 67.6, 68.9, 69.8, 71.3, 72.4, 73.2, 73.4, 73.9, 74.3, 74.5, 75.0, 76.0, 77.3, 78.1, 79.6, 79.9, 92.7, 99.3, 100.6, 101.0, 126.8-128.7, 129.8, 137.6, 137.7, 138.2, 138.3, 138.4, 140.5, 141.0, 165.2. LRMS (ESI) calcd for $C_{112}H_{124}N_2O_{24}S_2SiNa^+$ $[M+Na]^+$ 1995.8, found 1995.8.



Pentasaccharide 6: 6 was prepared using same procedure as the synthesis of **3**. White solid (80 mg, 74%). $[\alpha]_D^{25}$ 51.0 (*c* 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.07 (s, 3 H), -0.02 (s, 3 H), 0.80 (s, 9 H), 4.95 (s, 1 H), 4.99 (s, 1 H), 5.25 (s, 1 H), 5.54 (dd, *J* = 9.5, 2.5 Hz, 1 H), 5.58 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -4.6, 14.0, 17.9, 20.9, 22.5, 25.7, 31.4, 57.8, 58.6, 60.2, 67.7, 68.9, 69.6, 70.1, 71.5, 72.5, 72.9, 73.2, 73.9, 74.4, 74.8, 75.2, 75.9, 76.4, 77.3, 79.1, 92.7, 97.9, 99.4, 101.1, 126.9-129.5, 129.6, 137.3, 137.7, 138.2, 138.4, 141.1, 165.2, 165.5, 166.1. LRMS (ESI) calcd for $C_{146}H_{154}N_2O_{31}S_2SiNa^+$ $[M+Na]^+$ 2546.0, found 2545.9.

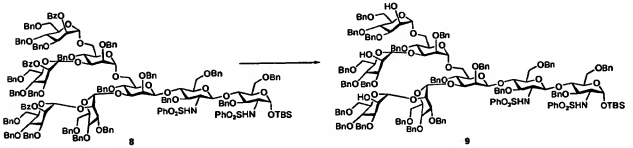


Pentasaccharide triol 7: To a solution of **6** (80 mg, 0.032 mmol) in MeOH (2 mL) was added sodium methoxide in MeOH (25%, 0.1 mL) and stirred for 12 h and quenched with NH_4Cl saturated aqueous solution and concentrated. The residue was dissolved in EtOAc and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by PTLC using pentane/ether (1/3) as the eluent to afford **7** as a white solid (64 mg, 91%). $[\alpha]_D^{25}$ 121.8 (*c* 0.16, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.05 (s, 3 H), 0.93 (s, 9 H), 4.92 (s, 1 H), 5.06 (d, J = 1.8 Hz, 1 H), 5.14 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -4.6, 14.0, 17.9, 20.9, 25.7, 57.8, 58.4, 60.2, 65.9, 68.4, 69.6, 71.6, 71.9, 72.4, 72.6, 73.1, 73.2, 73.3, 73.9, 74.1, 74.4, 74.7, 74.8, 74.9, 75.3, 75.8, 76.1, 76.4, 77.3, 78.3, 79.1, 79.6, 80.6, 92.7, 97.2, 100.5, 101.2, 101.3, 126.8-128.6, 137.6, 137.8, 137.9, 138.2, 138.3, 138.6, 140.5, 141.1. LRMS (ESI) calcd for $C_{125}H_{142}N_2O_{28}S_2SiNa^+$ [M+Na^+] 2233.9, found 2233.9.

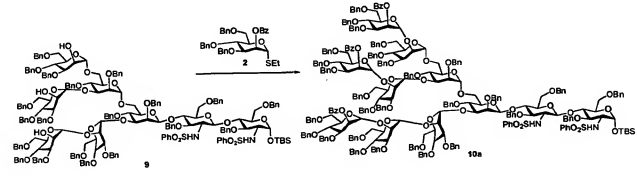


Octasaccharide 8: 8 was prepared following the same protocol as used for **3** using thiol mannoside donor **2** as excess (10 eq.) White solid: (61 mg, 55%). $[\alpha]_D^{25}$ 32.8 (*c* 0.15, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.05 (s, 3 H), 0.88 (s, 9 H), 4.79 (s, 1 H), 4.88 (s, 1 H), 5.01 (s, 1 H), 5.06 (s, 1 H), 5.23 (s, 1 H), 5.58 (s, 1 H), 5.62 (s, 1 H), 5.66 (s, 1

H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -3.9, 14.7, 18.5, 21.5, 26.3, 30.2, 58.4, 59.1, 60.9, 66.5, 69.0, 69.3, 69.4, 70.3, 71.3, 72.0, 72.2, 72.6, 72.8, 73.6, 73.7, 73.8, 74.0, 74.6, 75.1, 75.3, 75.5, 75.7, 78.4, 78.9, 80.4, 82.2, 93.3, 98.2, 98.9, 99.7, 101.2, 101.8, 102.8, 126.9-130.5, 138.5, 138.6, 138.8, 139.0, 139.1, 139.6, 141.2, 165.8, 165.9. LRMS (ESI) calcd for $C_{227}H_{238}N_2O_{46}S_2SiNa_2$ $[M+2Na]^{2+}$ 1932.8, found 1933.0.



Octasaccharide triol 9: The synthesis of 9 follows the synthetic procedure of **7**. White solid (46 mg, 87%). $[\alpha]_D^{25}$ 280.0 (*c* 0.12, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.08 (s, 3 H), -0.03 (s, 3 H), 0.80 (s, 9 H), 4.92 (s, 1 H), 4.94 (s, 1 H), 4.97 (s, 1 H), 5.03 (s, 1 H), 5.07 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.1, 18.0, 22.7, 25.8, 29.3, 29.7, 31.9, 58.0, 58.6, 65.5, 66.3, 67.7, 68.4, 68.5, 68.8, 71.1, 71.2, 71.7, 71.8, 72.0, 72.3, 72.9, 73.2, 73.3, 73.5, 74.1, 74.2, 74.3, 74.5, 74.8, 74.9, 75.0, 79.4, 81.8, 92.8, 97.4, 100.0, 100.7, 100.9, 101.3, 102.9, 127.1-128.8, 138.0-138.5, 140.7, 141.3. LRMS (ESI) calcd for $C_{206}H_{226}N_2O_{43}S_2SiNa_2$ $[M+2Na]^{2+}$ 1776.7, found 1776.7.



Undecasaccharide 10a: The synthesis of **10a** follows same synthetic procedure as **8**. **10a**, white solid (81 mg, 51%). $[\alpha]_D^{25}$ 73.8 (*c* 0.09, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ

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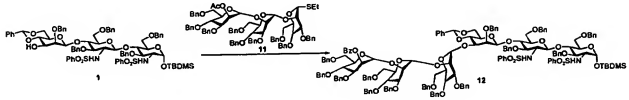
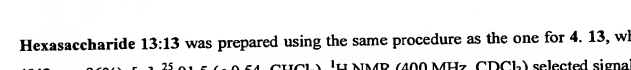
-0.05 (s, 3 H), -0.00 (s, 3 H), 0.82 (s, 9 H), 5.00-5.20 (m, 7 H), 5.65-5.68 (m, 3 H). LRMS (ESI) calcd for $C_{308}H_{322}N_2O_{61}S_2SiNa_2$ $[M+2Na]^{2+}$ 2581.1, found 2581.3.



Hexasaccharide 12: To a mixture of **1** (35 mg, 0.024 mmol), **11** (51 mg, 0.037 mmol) and molecular sieves in CH_2Cl_2 (2 mL) was added di-*tert*-butylpyridine (DTBP) (0.019 mL, 0.085 mmol) at -40 °C and stirred for 1 h at -40 °C. MeOTf (0.011 mL, 0.096 mmol) was added and the reaction mixture was warmed up to r.t. and stirred for 12 h before quenched with triethylamine, filtered through celite, washed with $NaHCO_3$ saturated aqueous solution, brine, dried over anhydrous $MgSO_4$ and filtered. The organic layer was concentrated and residue purified by PTLC using pentane/ether (1/1.3) as the eluent to afford **12** as a white solid (47 mg, 47%). $[\alpha]_D^{25}$ 41.4 (*c* 0.65, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.91 (s, 9 H), 4.97 (s, 1 H), 5.11 (s, 1 H), 5.21 (s, 1 H), 5.25 (s, 1 H), 5.28 (s, 1 H), 5.52 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.2, 18.0, 21.0, 21.1, 25.8, 58.0, 58.8, 60.4, 67.0, 68.6, 68.7, 69.8, 71.6, 71.9, 72.1, 72.9, 73.3, 73.7, 73.8, 74.4, 74.6, 75.1, 75.4, 75.5, 76.0, 77.4, 78.3, 78.8, 80.2, 92.9, 99.5, 99.9, 100.2, 100.8, 101.0, 101.1, 125.9, 127.1-128.5, 137.9, 138.1, 138.4, 138.6, 138.8, 140.7, 170.0, 171.1. LRMS (ESI) calcd for $C_{161}H_{176}N_2O_{34}S_2SiNa_2$ $[M+2Na]^{2+}$ 1409.6, found 1409.4.



Hexasaccharide 13:**13** was prepared using the same procedure as the one for **4**. **13**, white solid (542 mg, 86%). $[\alpha]_D^{25}$ 91.5 (*c* 0.54, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.02 (s, 3 H), 0.04 (s, 3 H), 0.87 (s, 9 H), 5.01 (s, 1 H), 5.06 (s, 1 H), 5.09 (s, 1 H), 5.15 (s, 1 H), 5.49 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.2, 18.0, 21.0, 21.1, 21.4, 25.8, 58.0,

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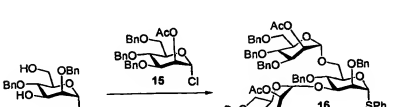
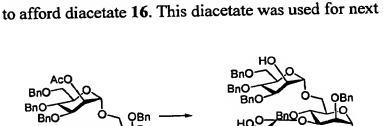
Client Reference No.: SK-1062-PRO3

58.3, 60.4, 61.3, 67.6, 68.6, 69.9, 71.8, 72.0, 72.1, 73.1, 73.3, 73.5, 74.1, 74.5, 74.6, 74.8, 75.1, 76.1, 76.2, 78.2, 78.7, 79.8, 81.1, 92.8, 99.4, 100.5, 100.7, 101.0, 101.3, 125.3, 127.0-128.5, 137.9, 138.0, 138.4, 138.5, 138.6, 138.7, 140.7, 170.1. LRMS (ESI) calcd for $C_{161}H_{178}N_2O_{34}S_2SiNa_2$ $[M+2Na]^{2+}$ 1410.6, found 1410.4.



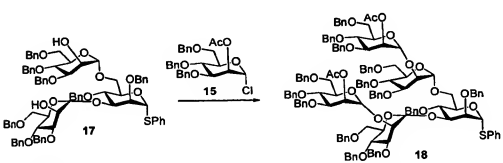
Tirsaccharide 16: To a 25 mL flask containing donor **15** (169 mg, 0.332 mmol) and acceptors **14** (37 mg, 0.083 mmol) (dried azeotropically with toluene) in 1.5 mL dichloromethane was added activated MS 4Å and the mixture was stirred for 1 h at room temperature. In a separate flask, AgOTf (0.087 gm, 0.332 mmol) and DTBP (0.078 mL, 0.347 mmol) in 1.5 mL dichloromethane were stirred with MS 4Å. After one hour the flask containing the AgOTf / DTBP was cooled to -10 °C and the solution containing mixture of donor and acceptor was added over 5 minutes. The solution was stirred in dark with warming up to room temperature over 18 hr. The reaction mixture was diluted with ethyl acetate and was added aqueous saturated $NaHCO_3$ solution. After stirring for 10 minutes, the reaction mixture was filtered through bed of Celite and the filtrate was washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (10% ethyl acetate/toluene) to afford diacetate **16**. This diacetate was used for next step without further purification.



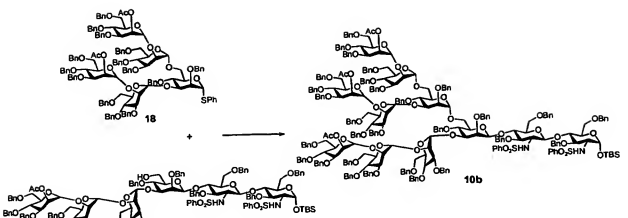
Trisaccharide diol 17:

16 was dried azeotropically with toluene and dissolved in 2 mL of anhydrous methanol under argon. Sodium methoxide (25% by weight in methanol, 100 µL) was added and the reaction mixture was stirred for 12 h. Solid ammonium chloride was added and the mixture was stirred

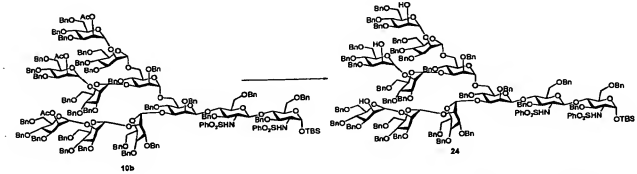
for 20 min. The reaction mixture was carefully evaporated to solid residues, and the residues were dissolved in ethyl acetate and washed with brine. Evaporation of ethyl acetate layers provided crude products, which was purified by silica gel column chromatography (10% ethyl acetate/dichloromethane) to yield diol **17** in 50% over two steps. $[\alpha]_D^{25}$ +53.1 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.94 (bs, 1H), 5.17 (bs, 1H), 5.44 (bs, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.68, 138.66, 138.4, 138.1, 138.06, 138.03, 134.9, 131.0, 129.3, 128.72, 128.70, 128.67, 128.61, 128.49, 128.47, 128.16, 128.13, 128.10, 128.01, 127.96, 127.88, 127.86, 127.83, 127.82, 127.79, 127.76, 127.71, 127.35, 99.9, 85.3, 80.5, 80.2, 79.6, 75.3, 75.15, 75.07, 74.6, 74.4, 73.8, 73.5, 72.7, 72.3, 72.2, 71.8, 71.7, 71.3, 69.5, 68.94, 68.90, 68.2, 66.4. LRMS (ESI) calcd for $C_{80}H_{84}O_{15}SNa^+$ $[M+Na]^+$ 1339.6, found 1339.5.



Pentasaccharide 18: To a mixture of **17** (208 mg, 0.158 mmol), **15** (332 mg, 0.631 mmol), molecular sieves, DTBP (0.088 mL, 0.347 mmol) in CH_2Cl_2 (13 mL) was added AgOTf (166 mg, 0.646 mmol) at 0 °C. The mixture was stirred for 18 h at r.t. and quenched with triethylamine, filtered, diluted with EtOAc, washed with $NaHCO_3$ saturated aqueous solution, brine, dried over anhydrous $MgSO_4$ and filtered. The organic layer was concentrated and residue purified by PTLC using pentane/ether (2/1) as the eluent to afford **18** as a white solid (310 mg, 87%). $[\alpha]_D^{25}$ 443.4 (*c* 0.49, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 2.10 (s, 3 H), 2.11 (s, 3 H), 4.88 (s, 1 H), 5.02 (s, 1 H), 5.04 (s, 1 H), 5.21 (s, 1 H), 5.51 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 20.9, 21.0, 21.1, 29.6, 44.6, 60.3, 66.6, 68.7, 68.8, 71.5, 71.7, 71.8, 72.0, 72.1, 73.1, 73.3, 73.4, 74.1, 74.2, 74.4, 74.6, 74.7, 75.0, 75.2, 78.0, 78.1, 79.2, 80.3, 84.8, 89.8, 95.4, 99.0, 99.4, 99.5, 101.2, 116.9, 125.1, 127.1-128.4, 129.1, 130.8, 138.0-138.6, 146.8, 168.3, 170.0. LRMS (ESI) calcd for $C_{138}H_{144}O_{27}SNa^+$ $[M+Na]^+$ 2288.0, found 2287.9.

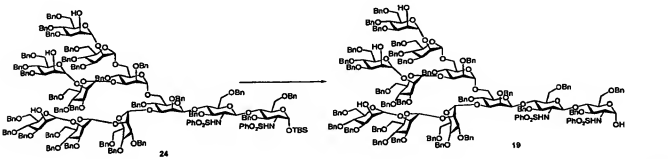


Undecasaccharide 10b: The preparation of **10b** from **18** and **13** follows the same procedure as the one used for **3**. **10b**, white solid (529 mg, 63% yield, 85% based on recovered starting material). $[\alpha]_D^{25}$ 214.3 (*c* 0.23, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.07 (s, 3 H), 0.15 (s, 3 H), 0.90 (s, 9 H), 2.01 (s, 3 H), 2.10 (bs, 6 H), 5.05 (bs, 1 H), 5.07 (bs, 1 H), 5.10 (bs, 1 H), 5.12 (bs, 1 H), 5.13 (bs, 1 H), 5.15 (bs, 1 H), 5.23 (bs, 1 H), 5.51 (bs, 1 H), 5.54 (bs, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 1.0, 14.2, 18.0, 21.0, 21.1, 21.2, 25.8, 29.7, 58.0, 58.6, 60.4, 68.6, 68.7, 68.8, 71.8, 72.2, 72.3, 73.0, 73.1, 73.2, 73.3, 74.2, 74.5, 74.8, 75.0, 75.1, 78.2, 78.3, 78.4, 79.4, 92.8, 99.3, 99.5, 100.7, 101.6, 102.3, 127.3-128.4, 138.1-138.7, 140.7, 141.3, 170.0, 170.1, 170.15. LRMS (ESI) calcd for $C_{293}H_{316}N_2O_{61}S_2SiNa_2$ $[M+2Na]^{2+}$ 2488.0, found 2488.0.

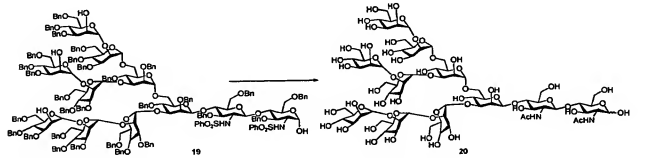


Undecasaccharide triol 24: **24** was prepared using the same procedure as described for **7**. **24**, white solide (468 mg, 96%). $[\alpha]_D^{25}$ 214.3 (*c* 0.23, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.03 (s, 3 H), 0.05 (s, 3 H), 0.90 (s, 9 H), 5.07 (s, 1 H), 5.08 (s, 1 H), 5.13 (s, 1 H), 5.18 (s, 1 H), 5.21 (s, 1 H), 5.30 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.5, 0.0, 14.1, 18.0,

21.0, 25.8, 29.6, 57.9, 58.6, 60.3, 67.6, 68.4, 68.6, 68.7, 68.9, 71.5, 71.9, 72.0, 72.3, 73.0, 73.1-73.5, 74.2, 74.5, 74.7, 74.9, 75.0, 75.2, 79.9, 80.0, 92.7, 99.4, 100.2, 100.7, 101.1, 101.5, 102.3, 126.7-128.7, 138.1-138.8, 140.7, 141.3. LRMS (ESI) calcd for $C_{287}H_{310}N_2O_{58}S_2SiNa_2$ $[M+2Na]^{2+}$ 2425.0, found 2425.2.



Undecasaccharide tetraol 19: To a solution of **24** (468 mg, 0.097 mmol) in HAc (1.0 M in THF, 2.5 mL) was added TBAF (1.0 M in THF, 2.5 mL) and the reaction mixture was stirred for 1 h before additional HAc (1.0 M in THF, 5.0 mL) was added. The mixture was concentrated and residue purified by column chromatography using 2.5% MeOH in CH_2Cl_2 as the eluent to afford **19** as a white solid (460 mg, 98%). $[\alpha]_D^{25}$ 121.7 (*c* 0.32, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.95 (bs, 2 H), 5.08 (s, 1 H), 5.20 (bs, 2 H), 5.24 (s, 1 H), 5.27 (s, 1 H). LRMS (ESI) calcd for $C_{281}H_{296}N_2O_{58}S_2Na_2$ $[M+2Na]^{2+}$ 2367.9, found 2367.6.



Glycan 20: To a solution of sodium (101 mg, 4.391 mmol) in 15 mL liquid ammonia was added **19** (95 mg, 0.020 mmol) in THF (4 mL) at -78 °C and the reaction mixture was stirred for 2 h at -78 °C. The reaction was quenched with solid NH_4Cl at -78 °C and then warmed up to r.t. while argon was blowing through the reaction flask to evaporate all liquid. The residue was dried on vaccum for 2 h and dissolved in saturated $NaHCO_3$ aqueous solution (2 mL) and cooled to 0 °C. Ac_2O (0.1 mL) was then added at 0 °C and the ice bath was then removed and 5 min later

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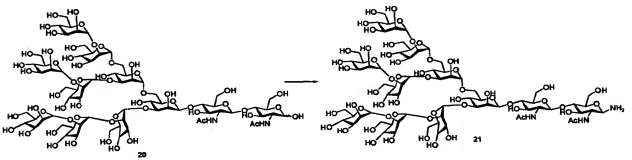
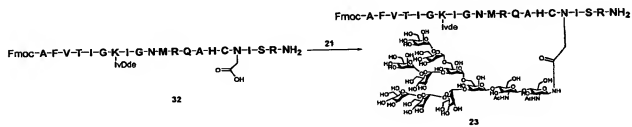
additional Ac_2O (0.05 mL) was added. 30 min later, low resolution mass spectrum showed reaction is complete. The reaction mixture was loaded on to a Bio-Gel P-2 column (BIO-RAD, catalog number 150-4134, molecular cutoff 2000) using water as the eluent to remove salt and small molecular weight compounds. The fraction containing desired material (illustrated by MassSpectrum) was conbined and lyophilized to afford glycan **20** as a white solid (33 mg, 87% from **19**). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 5.07 (bs, 2 H), 5.08 (s, 1 H), 5.13 (s, 1 H), 5.33 (s, 1 H), 5.36 (s, 1 H), 5.40 (s, 1 H). LRMS (ESI) calcd for $C_{70}H_{118}N_2O_{56}Na^+$ $[M+Na]^+$ 1905.6, found 1905.6.



Glycosylamine 21: A solution of **20** (33 mg, 0.018 mmol), NH_4Cl (10 g) in 30 mL water was heated to 40 °C for 2 days and Mass spectrum indicated that reaction is complete. So the reaction mixture was frozen and lyophilized. The residue was dissolved in 20 mL water, frozen and lyophilized again. This process was repeated until the weight of the residue is constant (36 mg). LRMS (ESI) calcd for $C_{70}H_{119}N_3O_{55}Na^+$ $[M+Na]^+$ 1904.7, found 1904.8.



GP120 glycopeptide 23: A solution of peptide acid **32** (21 mg, 0.008 mmol), HATU (6 mg, 0.016 mmol), diehtylpropylamine (DIEPA) (2 μL, 0.011 mmol) in DMSO (150 μL) was stirred for 5 min and transferred to the flask containing **21** (5 mg, 0.002 mmol) and the reaction mixture was stirred for 2 h. Additional DIEPA was added (0.6 μL at 4 h and 0.6 μL at 6 h). At 7 h, a

mixture of hydrazine, piperidine and DMF (volume ratio: 5:15:85, 0.2 mL) was added and the reaction mixture was stirred for 5 min and TFA in water (10%, 0.55 mL) was added and stirred for 30 min. The crude solution was purified by HPLC using a Varian C18-DYNAMAX-60 Å column. HPLC Conditions: 10%B to 50%B over 50 min, UV 214 nM (A: 0.05% TFA in water; B: 0.04% TFA in CH_3CN). Retention time: 19.8 min. The fraction containing **23** was lyophilized to give **23** as a white solid (1.7 mg, 16% from **20**). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.99 (s, 1 H), 5.16 (s, 1 H), 5.19 (s, 1 H), 5.25 (s, 1 H), 8.29 (s, 1 H). LRMS (ESI) calcd for $C_{164}H_{275}N_{35}O_{80}S_2Na_3$ $[M+3Na]^{3+}$ 1360.6, found 1360.7; calcd for $C_{164}H_{275}N_{35}O_{80}S_2Na_4$ $[M+4Na]^{4+}$ 1020.7, found 1020.6.

Reference:

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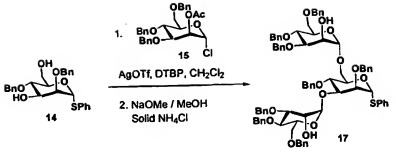
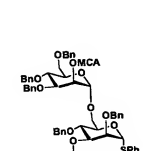


Into a 25 mL flask containing donor **15** (0.169 gm, 0.332 mmol) and acceptors **14** (0.037 gm, 0.083 mmol) (azeotropically dried with toluene) in 1.5 dichloromethane was added activated MS 4A and the mixture was stirred for 1 hr at room temperature. In a separate flask, AgOTf (0.087 gm, 0.332 mmol) and DTBP (0.078 mL, 0.347 mmol) in 1.5 mL of dichloromethane were stirred with MS 4A. After stirring for 1 hr, the flask containing the AgOTf / DTBP was cooled to – 10 °C and the solution containing mixture of donor and acceptor was added over 5 minutes. The solution was stirred in dark with gradual warming up to room temperature over 24 hr. The reaction mixture was diluted with ethyl acetate and was added aqueous saturated $NaHCO_3$. After stirring for 10 minutes, the reaction mixture was filtered through bed of Celite and the filtrate was washed with water, then with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (10% ethyl acetate / toluene) to afford semi pure trimer diacetate. This diacetate was dried azeotropically with toluene and dissolved in 2 mL of anhydrous methanol under argon. Sodium methoxide, 25% by weight in methanol (100 μL) was added and the reaction mixture was stirred for 12h. Solid ammonium chloride was added and the resulting solution was stirred for 20 min. The reaction mixture was carefully evaporated to solid residues, and the solid residues were washed with ethyl acetate. Evaporation

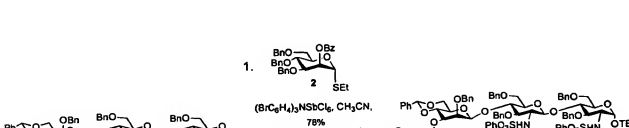
of ethyl acetate layer provided crude product, which was purified by silica gel column chromatography (10% ethyl acetate / dichloromethane) to yield diol **17** in 65% yield (over two steps. [α] + 53.1 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) δ 7.33-7.03 (45H, m, aromatic), 5.44 (1H, br-s), 5.17 (1H, br-s), 4.94 (1H, br-s), ; ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 138.68, 138.66, 138.4, 138.1, 138.06, 138.03, 134.9, 131.0, 129.3, 128.72, 128.70, 128.67, 128.61, 128.49, 128.47, 128.16, 128.13, 128.10, 128.01, 127.96, 127.88, 127.86, 127.83, 127.82, 127.79, 127.76, 127.71, 127.35, 99.9, 85.3, 80.5, 80.2, 79.6, 75.3, 75.15, 75.07, 74.6, 74.4, 73.8, 73.5, 72.7, 72.3, 72.2, 71.8, 71.7, 71.3, 69.5, 68.94, 68.90, 68.2, 66.4. ESI-MS calcd for $C_{80}H_{84}O_{15}S$ Na $[M+Na]^{1+}$ m/z = 1339.5: found 1339.5



22

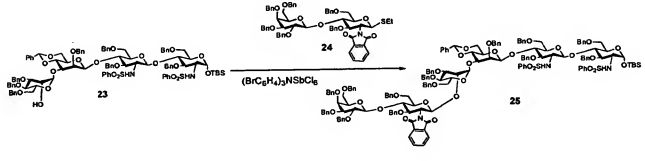
Into a 25 mL flask containing **17** (0.099 gm, 0.0689 mmol, azeotropically dried with toluene) in 0.4 mL mL of dry dichloromethane under argon and cooled to 0 °C. Pyridine (55 μL, mmol, 6.8 mmol) and chloroacetic anhydride (0.047 gm, .0275 mmol), were added successively and resulting reaction mixture was stirred for 2 h at 0 °C, and then diluted with ethyl acetate, washed two times with 0.5N HCl, water, sat $NaHCO_3$, brine, and dried with $MgSO_4$. Evaporation of

ethyl acetate layer followed by silica gel column chromatography (20 ethyl acetate in hexanes) provided 0.166 gm (79% yield) of **22**. R_f 0.33 (20% ethyl acetate in hexanes). [α] + 58.2 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) δ 7.30-7.03 (45H, m, aromatic), 5.47 (2H, m), 5.41 (1H, br-s), 5.15 (1H, s), 4.88 (1H, s), 4.75 (2H, t, J = 10.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.88, 166.78, 138.57, 138.51, 138.3, 137.79, 137.76, 137.74, 134.7, 130.9, 129.3, 128.7, 128.6, 128.59, 128.51, 128.48, 128.43, 128.30, 128.10, 128.0, 127.97, 128.86, 127.78, 127.75, 127.74, 127.7, 127.4, 99.5, 97.9, 84.9, 79.0, 78.1, 77.7, 75.4, 75.2, 75.1, 74.3, 74.1, 73.7, 73.5, 72.43, 72.37, 72.29, 71.78, 71.69, 70.7, 70.4, 69.0, 68.7, 66.8, 41.2, 41.0



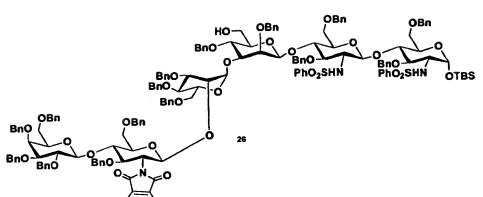
Into a 25 mL flask containing donor **2** (125 mg, 0.0696 mmol, azeotropically dried with toluene) and 4A molecular sieves in dry acetonitrile were stirred for 1 hr under argon. Tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (140 mg, promoter) and then a solution of acceptor **1** (100 mg, 0.0696 mmol) were added slowly while cooling the flask at 15 °C. After stirring for 15 min, another portion of tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (46 mg) was added and the reaction mixture was warmed to room temperature and stirred for 3 hr. Freshly distilled triethyl amine (1.5 mL) was added to neutralize the reaction. The reaction mixture was filtered through a bed of Celite and concentrated. The crude product was purified by silica gel column chromatography to afford tetrasaccharide (0.110 gm). R_f 0.65 (20% ethyl acetate in toluene). Under argon this material was

dissolved in mixture of dry methanol (2 mL) and dichloromethane (1.5 mL). Sodium methoxide, 25% by weight in methanol (0.038 mL) was added and stirred for 12 hr. Solid ammonium chloride was added and the mixture was evaporated to dryness. The solid residue was washed several times with ethyl acetate and concentrated. Purification by silica gel column chromatography afforded the 0.092 gm (89% yield) of **23**. R_f 0.42 (40% ethyl acetate in hexanes). [α] – 8.8 (c 1, $CHCl_3$);^1H – NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.75 (2H, d, J = 7.6 Hz), 7.72 (2H, d, J = 6.8 Hz), 5.41 (1H, br-s), 5.26 (1H, d, J = 2.0 Hz), 5.11 (1H, d, J = 2.4 Hz), 3.14 (1H, m), 3.0 (2H, m), 1.57 (1H, br-s), 0.908 (9H, s), 0.09 (3H, s), 0.03 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 141.7, 140.9, 138.7, 138.65, 138.61, 138.4, 138.0, 137.9, 137.8, 137.5, 132.6, 132.4, 129.5, 129.1, 129.0, (128.9-127.6), 127.4, 127.3, 127.2, 126.2, 101.5, 101.3, 101.1, 100.4, 93.0, 80.3, 80.0, 76.2, 75.8, 75.6, 75.5, 75.2, 74.8, 74.4, 73.96, 73.87, 73.6, 72.2, 72.0, 69.9, 69.3, 68.8, 68.5, 68.2, 67.8, 67.2, 37.5, 33.8, 33.6, 32.1, 30.3, 30.2, 29.9, 29.5, 29.1, 27.3, 26.9, 26.0, 23.4, 22.9, 19.9, 18.2, 14.4, 14.3, 7.6, - 4.2, - 5.4;



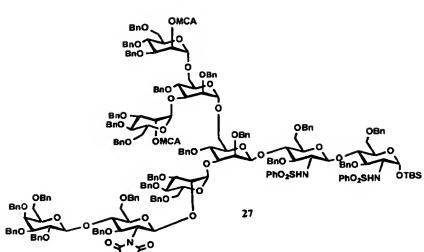
Into a 25 mL round-bottomed flask containing acceptor **23** (0.100 gm, 0.0535 mmol, azeotropically dried with toluene) in 1 mL dry acetonitrile was added 4A activated molecular

sieves and was stirred at room temperature under argon for 1 hr. Similarly the donor **24** (0.1498 mmol, 0.158 gm) and 4A molecular sieves in 1 mL dry acetronitrile were stirred at room temperature for 1 hr. The flask containing donor was cooled to 15 °C and 0.091 gm of tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (promoter) was added followed by the solution of acceptor. The reaction mixture was stirred at 15 °C for 20 min and then additional 0.031 gm of promoter was added. The cooling bath was removed and the reaction mixture was stirred for 3 hr. The reaction mixture was cooled to 0 °C and triethyl amine (1 mL) was added. After stirring 10 min at 0 °C, the reaction mixture was warmed to room temperature and stirred for additional 10 min. Filtering through a pad of Celite and evaporation of the filtrate afforded the crude product, which was purified by preparative TLC (first using 5% ethyl acetate in dichloromethane, then 30% ethyl acetate in hexanes) to afford .091 gm of **25** (60% yield), [α] – 16.6 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.72 (2H, d, J = 7.2 Hz), 7.67 (2H, d, J = 7.2 Hz), 5.21 (1H, br-s), 5.07 (1H, br-s), 5.01 (1H, br-s), 4.98 (1H, d, J = 8Hz), 2.99 (1H, m), 2.89 (1H, t, J = 8.4 Hz), 2.80 (1H, m), 2.64 (2H, m), 0.875 (9H, s), 0.07 (3H, s), 0.05 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 140.5, 139.7, 138.04, 138.01, 137.7, 137.69, 137.63, 137.59, 137.56, 137.47, 137.41, 137.3, 137.0, 136.8, 136.5, 136.4, 132.4, 131.3, 131.1, 130.9, 128.4, 128.1, 127.8, (127.5-126.1), 125.8, 125.3, 122.4, 122.0, 102.3, 100.3, 99.9, 99.4, 96.5, 94.7, 91.8, 81.5, 78.9, 77.6, 77.36, 77.31, 75.1, 74.4, 74.2, 74.1, 73.9, 73.8, 73.6, 73.5, 73.1, 72.8, 72.7, 72.4, 72.2, 71.9, 71.8, 71.6, 71.5, 71.2, 57.8, 56.9, 51.3, 28.7, 24.8, 16.9, 7.6, - 5.4, - 6.6



To the azeotropically dried **25** (0.307 gm, 0.107 mmol) in 25 mL round bottomed flask was added 8 mL of BH_3.THF (1 molar) and stirred for 5 minutes at room temperature. The reaction mixture was cooled to 0 °C and 0.35 mL of $n$$Bu_2OTf$ (1 molar in CH_2Cl_2) was added. The resulting reaction mixture was stirred at 0 °C for 9 hr. Freshly distilled triethyl amine (0.492 mL) was added and followed by careful addition of methanol until the evolution of H_2 had ceased. The reaction was evaporated to dryness, twice codistilled from methanol to afford the crude product as clear oil. Purification by silica gel column chromatography (30% ethyl acetate in hexanes) provided the **26** in 75% yield (0.231 gm).

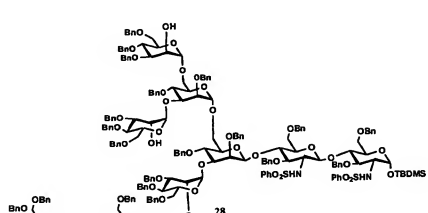
$[\alpha]$ – 7.0 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.74 (2H, d, J = 7.2 Hz), 7.69 (2H, d, J = 6.8 Hz), 5.07 (2H, m), 2.96 (1H, m), 2.75 (2H, m), 0.90 (9H, s), 0.07 (3H, s), 0.02 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 168.5, 167.7, 141.4, 140.9, 139.3, 139.2, 138.97, 138.91, 138.73, 138.66, 138.64, 138.58, 138.50, 138.45, 138.27, 138.1, 137.8, (129.2-126.9), 123.5, 123.4, 103.3, 101.0, 100.9, 99.2, 96.5, 92.9, 82.6, 80.1, 79.9, 78.7, 78.4, 76.5, 76.0, 75.9, 75.4, 75.1, 74.9, 74.8, 74.68, 74.63, 74.49, 74.34, 74.29, 74.24, 73.84, 73.77, 73.6, 73.45, 73.3, 73.2, 73.0, 72.8, 72.7, 61.4, 60.6, 58.6, 59.2, 55.8, 26.0, 21.3, 19.3, 18.2, 14.4, 14.1, - 4.2, - 5.4;



27

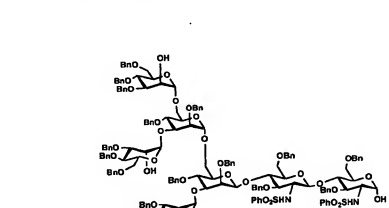
Into a 5 mL v vial were taken azeotropically dried donor **22** and acceptor in **26** mL anhydrous acetonitrile and activated 4A MS was added. The resulting reaction mixture was stirred under argon for 1 hour at room temperature and then was cooled to 15 °C. At this point Tris (4-bromophenyl) aminium hexachloroantimonate $[(BrC_6H_4)_3NSbCl_6]$ was added to the reaction mixture. The cooling bath was removed and the reaction mixture was stirred at room temperature for 12 h or TLC indicated the disappearance of the acceptor. The reaction mixture was cooled to 0 °C and 2 mL triethyl amine was added and stirred for 30 minutes with gradual warming up to room temperature. The reaction mixture was filtered through a pad of Celite and concentrated to provide crude material, which was purified by preparative TLC (20x20cm x 1 mm thickness PK6F plates) using 40% ethyl acetate in hexanes to yield **27**. [α] + 9.4 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) (selected protons) δ 5.41 (1H, br-s), 5.32 (1H, br-s), 5.09 (1H, br-s), 4.97 (2H, m), 0.83 (9H, s), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz)□ 168.6, 167.6, 166.70, 166.67, 141.5, 140.9, 139.30, 139.23, 139.0, 138.9, (138.8-138.1), 137.89, 137.86, 137.6,

133.6, 132.5, 132.3, 132.0, (129.0-126.9), 126.7, 103.3, 101.7, 100.9, 99.3, 98.0, 97.8, 96.3, 92.9, 82.6, 81.3, (78.9-65.1), 58.6, 58.1, 55.8, 39.9, 39.8, 28.7, 24.8, 17.0, - 5.4, - 6.7.



To azeotropically dried **27** (100 mg, 0.023 mmol) in a v 5 mL v vial equipped with spin bar were added fresh toluene (2 mL) and *n*-butanol (4 mL). Ethylenediamine was added and the reaction mixture was heated at 90 o C for 18 hr. After cooling to room temperature, the reaction mixture was concentrated under *vacuo*. The crude product was dissolved in 5 mL of toluene and evaporated to dryness. Pyridine and acetic anhydride were added and the reaction mixture was stirred for 16 hr at room temperature. The reaction mixture was evaporated to dryness, twice from toluene, yielding foam with some solid. This material was dissolved in 5 mL of methanol and 2 mL of THF under argon and 0.35 mL of 25% sodium methoxide in methanol was added and the reaction mixture was stirred for 12 hr. Solid ammonium chloride was added and stirred for 30 min. Careful evaporation of this biphasic reaction mixture provided white solid residue, which was washed three times by ethyl acetate. Concentration of ethyl acetate layer yielded the crude product, which was purified by preparative TLC (10% ethanol in toluene) to afford **28** in

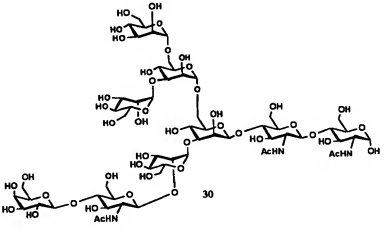
69% yield (0.064 gm). R_f = 0.67 (10 ethanol in toluene). [α] + 14.6 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 500 MHz) (selected protons) δ 7.65 (2H, d, J = 9 Hz), 7.60 (2H, d, J = 7.5 Hz), 5.22 (1H, d, J = 8 Hz), 5.10 (1H, br-s), 5.07 (2H, br-s), 3.06 (1H, m), 2.96 (1H, m), 2.24 (2H, d, J = 14.5 Hz), 1.68 (3H, s), 0.90 (9H, s), 0.07 (3H, s), 0.027 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz)δ 169.6, 141.5, 140.9, 139.6, 139.3, 139.1, 138.97, 138.95, 138.89, 138.7, 138.6, 138.5, 138.39, 138.36, 138.3, 138.2, 138.0, 137.7, 132.6, 132.4, 129.0, 128.9, (128.7-127.3), 127.2, 126.7, 103.0, 102.0, 100.9, 100.0, 99.9, 98.3, 97.793.0, 82.5, 81.4, 80.2, 80.1, 79.7, 79.4, 78.8, 78.6, 78.1, 77.9, 77.8, 76.6, 76.0, 75.3, 75.2, 74.9, 74.8, 74.7, 74.4, 74.38, 74.35, 74.2, 73.9, 73.7, 73.6, 73.5, 73.49, 73.45, 73.37, 73.2, 73.1, 72.0, 71.9, 71.8, 71.4, 71.4, 71.3, 71.1, 70.0, 69.7, 69.1, 68.9, 68.7, 68.4, 67.9, 67.8, 66.7, 65.7, 58.8, 58.2, 57.2, 26.0, 23.6, 18.2, 1.2, -4.2, 5.4.



29

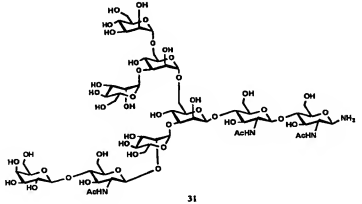
To the azeotropically dried **28** in a 25 mL round bottomed flask equipped with stir bar was added 0.5 mL 1M acetic acid in THF and the reaction mixture was cooled to 0 °C. To this ice cooled reaction mixture was added 0.5 mL TBAF (1M in THF). The cooling bath was removed and the reaction mixture was stirred for 3 hr. Additional 2 mL 1M acetic acid in THF was added and the

reaction mixture was stirred for 15 min. The reaction mixture was evaporated to dryness and the crude product was purified by preparative TLC (10% ethanol in toluene) to afford 0.055 gm (89% yield)of **29**. [α] + 9.40 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 500 MHz) (selected protons) δ 7.66 (2H, d, *J* = 8 Hz), 7.60 (2H, d, *J* = 7.6 Hz), 5.17 (1H, d, *J* = 7.2 Hz), 5.07 (1H, br-s), 5.01 (2H, m), 2.34 (1H, br), 2.18 (1H, br), 1.61 (s, 3H),



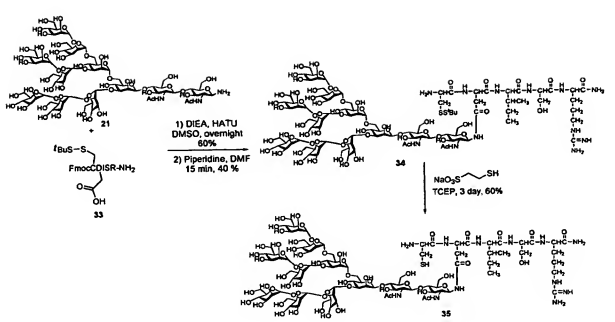
Into a three necked round bottomed flask, equipped with dry ice acetone condenser was condensed 15 mL ammonia under argon. Sodium metal (0.095 gm, 153 equiv.) was added in three portions. The resulting blue solution was stirred for 30 min at – 78 °C. The compound **29** (0.104 gm, 0.027 mmol) in 1.5 mL (3 x 0.5 mL) was added to the solution and the reaction mixture was stirred for 2 hr. Solid ammonium chloride (0.263 gm, 4.97 mmol) was added to quench the reaction and the reaction mixture was warmed to room temperature slowly. Evaporation of the residual liquid provided solid residue, which was dissolved in 5 mL pyridine. To this mixture was added acetic anhydride (3 mL) and DMAP (5 mg) and the resulting mixture was stirred with slowly warming to room temperature over 12 hr. The reaction mixture was evaporated to dryness and purified carefully by silica gel column chromatography to afford

peracetate. The peracetate in 5 mL methanol was added solution of NaOMe, 25% by weight in methanol (0.4 mL) and was stirred for 24 hr. The resulting cloudy solution was treated with water at 0 °C and stirred for another 24 hr. The reaction mixture was neutralized using Amberlyst – 15 acidic regin and evaporated to afford crude product, which was purified by size exclusion chromatography using Bio-Gel P2 regin yielding 30 mg of free glycan.



31

Free glycan (10 mg) in 15 mL of saturated ammonium bicarbonate was heated at 40 °C. Additional ammoniumhydrogen carbonate was added time to time to keep the solution saturated. After two days of stirring the content of the flask was shell frozen, lyophilized, dissolved in water (10 mL), lyophilized; this process was repeated until the white solid residue reached constant mass of 10 mg, which was used directly in the next step.



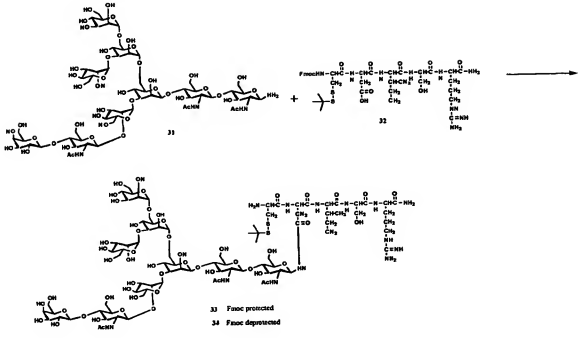
Glycopeptide 34:

A solution of acid **33** (6 mg, 0.007 mmol), HATU (5 mg, 0.013 mmol), DIEPA (1.7 μL, 0.012 mmol) in DMSO (0.1 mL) was stirred for 10 min and transferred to a falcon tube (25 mL) containing 4.2 mg of **21**. The solution was stirred for 2 h and additional DIEPA (1.2 μL) was added. The reaction mixture was purified by semiprep HPLC column (30 to 50%B over 20 min) to afford Fmoc-protected glycopeptide (3.6 mg, 60%). LRMS (ESI) calcd for $C_{111}H_{177}N_{12}O_{64}S_2Na^{++}$ $[M+H+Na]^{++}$ 1394.5, found 1394.5. This Fmoc-protected glycopeptide was dissolved in 0.4 mL pipyridine/DMF (1:4) solution and stirred for 15 min and quenched by THF/H_2O (10%) until the pH = 2~3. The crude mixture was purified on semiprep HPLC column (5 to 25%B over 20 min) to afford **34** (2 mg, 40%). LRMS (ESI) calcd for $C_{96}H_{167}N_{12}O_{62}S_2Na^{++}$ $[M+H+Na]^{++}$ 1283.5, found 1283.6. ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.99 (s, 1 H), 5.02 (s, 1 H), 5.16 (s, 1 H), 5.18 (s, 1 H), 5.25 (s, 1 H).

Glycopeptide 35:

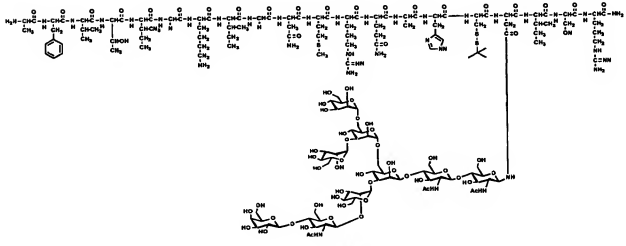
To a solution of **34** (2 mg, 0.0008 mmol) in phosphorous buffer (NaH_2PO_4 and Na_2HPO_4, pH=7.4, 0.5 mL) was added $HSCH_2CH_2SO_3Na$ (10 mg, 0.061 mmol) and stirred for 2 days. TCEP (30 mg, 0.104 mmol) was then added and the resulting solution was stirred for 1 h. The residue was purified on semiprep HPLC column (5 to 25%B over 20 min) to afford **35** (1.7 mg, 60%). LRMS (ESI) calcd for $C_{92}H_{160}N_{12}O_{62}S^{++}$ $[M+2H]^{++}$ 1228.5, found 1228.5. ^1H NMR (400

MHz, $CDCl_3$) selected signals: δ 4.90 (s, 1 H), 4.99 (s, 1 H), 5.15 (s, 1 H), 5.18 (s, 1 H), 5.25 (s, 1 H).



To a 15 mL polypropylene conical tube equipped with stir bar in 0.2 mL DMSO was added peptide **32** (11 mg, 3 equiv.) and HATU (15 mg, 5.9 equiv.). The solution was stirred for 1 min and was added diisopropyl ethyl amine (3.58 μL, 3 equiv.) and was stirred for another minute. This orange-yellow solution was transferred *via* 0.5 mL syringe to the flask containing glycosylamine **31** (11 mg). The conical tube was rinsed with additional 0.1 mL of DMSO and transferred to the flask containing glycosyl amine using the same 0.5 mL syringe. Monitoring by LCMS showed that no additional product formation after 6 hr. Purification of the reaction mixture by size exclusion chromatography provided the **33**. To this Fmoc protected **33** was added a 1 : 3 : 16 mixture of hydrazine : piperidine : DMF (200 μL). The resulting yellowish solution was stirred for 30 min before addition of a solution of TFA to bring the pH to 3. The

reaction mixture was purified by semiprep HPLC column (5 to 25%B over 25 min) to afford the Fmoc deprotected **34** in 30% yield. ^{1}H – NMR ($CDCl_3$, 500 MHz) (selected protons) δ 4.78 (2H, d, J = 12.4 Hz), 4.70 (1H, d, J = 9.6 Hz), 4.57 (2H, d, J = 13.2 Hz), 4.26 (4H, m), 4.14 (1H, d, J = 7.2 Hz), 1.01 (9H, s), 0.575 (6H, m).



35

Compound **35** was prepared similar to **34**.

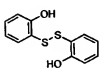
Experimental:

Reagents: All commercial materials were used as received unless otherwise noted. The following solvents were obtained from a dry solvent system and used without further purification: THF, diethyl ether, toluene, and DCM. Reagents were obtained from Aldrich or as noted, with the following exceptions: amino acids and resins for solid phase peptide synthesis were purchased from NovaBiochem; Biosynthesis grade DMF from EM Science; and all other solvents from Fisher Scientific (HPLC grade).

HPLC: All separations involved a mobile phase of 0.05% TFA (v/v) in water (solvent A)/0.0425% TFA in acetonitrile (solvent B). Preparative, semipreparative, and analytical HPLC separations were performed using a Rainin HXPL solvent delivery system equipped with a Rainin UV-1 detector and one of the following Dynamax-60Å C18 axial compression columns 250 mm in length equipped with a similarly packed guard column: 41.4 mm diameter (prep), 21.4 m diameter (semiprep), or 4.6 mm diameter (analytical). Separations were performed at flow rates of 48 mL/min (prep), 16 mL/min (semiprep), or 1 mL/min (analytical), and were monitored at a wavelength between 214 and 230 nm, depending on column loading. LCMS chromatographic separations were performed using a Waters 2695 Separations Module and a Waters 996 Photodiode Array Detector equipped with a Varian Microsorb C18 2 x 150 mm column at a flow rate of 0.2 mL/min.

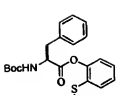
ESMS and LCMS: Electrospray mass spectroscopy and LCMS analyses were obtained on a Waters Micromass ZQ mass spectrometer in conjunction with the Waters HPLC apparatus described above.

NMR: ^{1}H and ^{13}C NMR spectra were recorded on Bruker instruments in $CDCl_3$, CD_3OD or D_2O at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C.

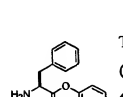


To a stirred, biphasic solution of 2-mercaptophenol (1.0 g, 7.9 mmol) in H_2O (5.1 mL) was added, drop wise, a solution of iodine (1.0 g 4.0 mmol) in methanol (3.5 mL). When the brown iodine color persisted the solution was diluted with ethyl acetate and water. The aqueous layer was removed and extracted with an additional portion of ethyl acetate. The combined organic layers were dried and washed with brine then dried (Na_2SO_4) and concentrated to give a brown oil which was used without purification (1.5 g). The product still contains iodine. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33-7.37 (m, 2H), 7.22-7.24 (m, 2H), 6.99-7.01 (m, 2H), 6.82-6.85 (m, 2H), 6.22 (brs, 2H). ^{13}C ($CDCl_3$, 125 MHz): δ 157.3, 136.6, 133.6, 121.4, 120.3, 116.1. ESI-MS: Calcd. for $C_{12}H_{10}O_2S_2$ $[M+NH_4]^+$ 267.8 Found: 267.8.

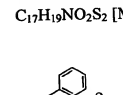
To a stirred solution of the disulfide (1.5 g crude, ~4 mmol) in CH_2Cl_2 (25 mL) was added ethyldisulfide (10.5 mL, 79.9 mmol) and then $BF_3 \cdot OEt_2$ (10.1 mL, 79.9 mmol). The reaction was stirred at room temperature for three hours and then carefully quenched by the addition of $NaHCO_3$. The organic layer was drained and the aqueous layer was extracted with an additional portion of CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated to give a yellow oil. Purification by silica gel chromatography (20% ethyl acetate in hexane) gave the desired product as a clear, slightly yellow oil (1.45 g, 99%).^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.48-7.51 (m, 1H), 7.28-7.32 (m, 1H), 6.99-7.01 (m, 1H), 6.86-6.9 (m, 1H), 6.34 (brs, 1H), 2.78 (q, J = 7.4 Hz, 2H), 1.35 (t, J = 7.4 Hz, 3 H). ^{13}C ($CDCl_3$, 125 MHz): δ 156.9, 135.2, 132.2, 121.0, 116.2, 32.4, 14.1. ESI-MS: Calcd. for $C_8H_{10}OS_2$ $[M+Na]^+$ 208.8 Found: 208.8.



To a well stirred solution of the phenol (1.45 g, 8 mmol) and Boc-Phe-OH (2.65 g, 10 mmol) in CH_2Cl_2 (25 mL) and THF (5 mL) was added EDCI (1.92 g, 10 mmol) and DMAP (98 mg, 0.8 mmol). The resulting solution was stirred are room temperature for 18 hr at which point the volatile materials were removed *in vacuo*. The resulting oil was taken up in EtOAc and washed with 1N HCl, H_2O, and then brine. The organic layer was dried (Na_2SO_4) and concentrated to give a slightly yellow oil. Purification by silical gel chromatography (30% ethyl acetate in hexane) gave a clear, colorless oil (3.5 g, >99%). The product contains a small amount of ethyl acetate, even after one day under high vacuum. ESI-MS: Calcd. for $C_{22}H_{27}NO_4S_2$ $[M+Na]^+$ 456.0 Found: 456.0.

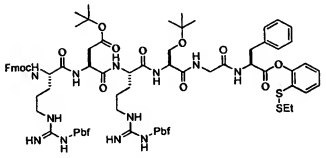


To a 50 mL polypropylene conical tube was added H_2O (5.4 mL), triethylsilane (2.6 mL, 16 mmol), and TFA (30 mL). The contents were thoroughly mixed and then added directly to the phenylalanine derivative (3.5 g, 8 mmol). The mixture was stirred for 15 min and then concentrated. H_2O was added and the mixture was again concentrated. This was repeated two more times at which point a white solid appeared at the end of concentration and the flask no longer smelled of TFA. The material was placed under high vacuum for 18 hours and then dissolved with a 30 % solution of acetonitrile in H_2O. The liquid was shell frozen and lyophilized to give a white powder. ESI-MS: Calcd. for $C_{17}H_{19}NO_2S_2$ $[M+H]^+$ 334.1 Found: 334.2.

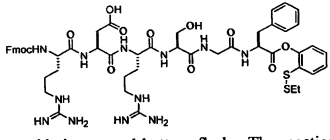


The phenylalanine derivative (15 mg, 45 μmol) and L-cysteine (6 mg, 49 μmol) were placed into a LCMS vial along with a flea-sized stirbar. In a second vial were mixed MESNa (25 mg, 150 μmol) and phosphate buffered saline (0.2M NaCl, 0.2M phosphate, pH=7.5, 2 mL). The MESNa solution was then added directly to the amino acids, and the reaction was monitored by LCMS. After two hours the reaction appeared to be complete and TCEP (129 mg, 450 μmol) was added. This was stirred for 1 hour and then injected directly onto the HPLC for purification. The desired compound was obtained as a white powder after lyophilization. ^1H NMR (D_2O): δ 7.36-7.45 (m, 3H), 7.31-7.33 (m, 2H), 4.63 (dd, J = 6.4, 5.0 Hz, 1H), 4.34 (dd, J = 7.2, 7.1 Hz, 1H), 3.29 (dd, J = 14.1, 7.1 Hz, 1H), 3.23 (dd, J =

14.1, 7.2 Hz, 1H), 2.99 (dd, J = 14.2, 5.0 Hz, 1H), 2.93 (dd, J = 14.2, 6.4 Hz, 1H). ESI-MS: Calcd. for $C_{12}H_{16}N_2O_3S$ $[M+H]^+$ 269.1, Found: 269.1



The peptide (127 mg, 0.089 mmol) and phenylalanine derivative (38 mg, 0.116 mmol) were dissolved in THF (0.16 mL) along with DMAP (1 mg, 0.009 mmol). CH_2Cl_2 (0.63 mL) was added and the mixture was stirred for 5 min. Solid EDCI (21 mg, 0.111 mmol) was added and the reaction was stirred at room temperature for 18 hr then quenched by the addition of 1N HCl and diluted with EtOAc. The aqueous layer was removed and extracted with a second portion of EtOAc. The combined organic layers were washed with water then brine, dired (Na_2SO_4) and concentrated to give a white powder. The material was purified by filtration through a small plug of silica gel (30% to 100% ethyl acetate in hexane) to give white solid (95 mg, 61%). ESI-MS: Calcd. for $C_{87}H_{114}N_{12}O_{18}S_4$ $[M+H]^+$ 1743.7, Found: 1743.7.



To a 50 mL polypropylene conical tube was added H_2O (0.7 mL), triethylsilane (0.3 mL, 1.9 mmol), phenol (678 mg, 0.082 mmol) and TFA (18 mL, 234 mmol). The contents were thoroughly mixed and then added directly to the peptide in a round bottom flask. The reaction was stirred at room temperature for three hours and then all volatile materials were removed *in vacuo*. Purification by direct injection on the HPLC (40-60%B over 20 min, rt = 15.4 min) to gave the desired material as a white powder after lyophilization (21 mg). ESI-MS: Calcd. for $C_{53}H_{66}N_{12}O_{12}S_2$ $[M+H]^+$ 1127.4, Found: 1127.4.

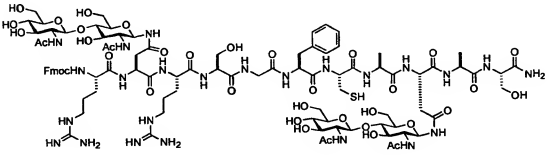
Typical aspartylation conditions:

Peptide (0.021 mmol) and HATU (0.042 mmol) were combined in a 50 ml conical tube and DMSO (0.17 mL) was added. After 5 min the resulting solution was added to a solution of the glycosylamine (0.23 mmol) in DMSO (0.17 mL). The solution was stirred for 10 min and then

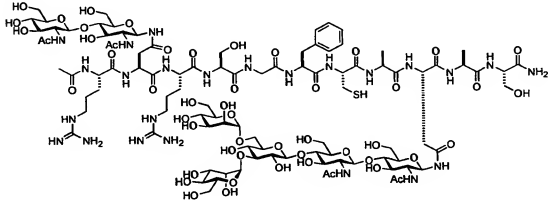
DIEA (3.7 μL, 0.21 mmol) was added. The reaction was monitored by LCMS and upon completion was injected directly on the HPLC for purification.

Typical ligation conditions:

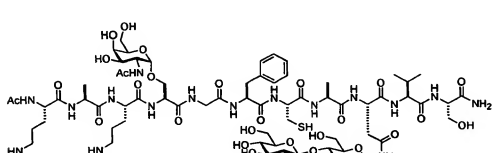
The two glycopeptide halves were placed in a LCMS vial along with a flea-sized stirbar. A stock solution of MESNa (18.3 mg, 111 mmol) in phosphate buffered saline (0.2M NaCl, 0.2M phosphate, pH=7.4, 1 mL) was made and of this, 600 μL was added to the glycopeptides. The reaction was monitored by LCMS and, once finished, TCEP (25 mg, 0.087 mmol) was added and the solution stirred for 2 hr then injected directly onto the HPLC.



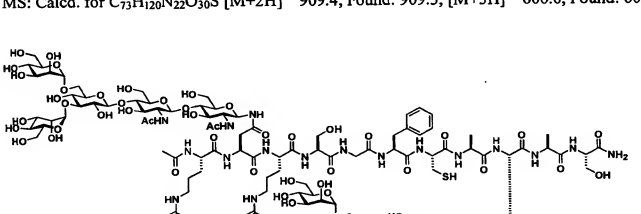
LCMS: 5-65%B over 20 min, rt = 15.17 min. HPLC: 25-55%B over 30 min, rt = 7.65 min. ESI-MS: Calcd. for $C_{93}H_{138}N_{24}O_{37}S$ $[M+2H]^{2+}$ 1108.5, Found: 1108.6, $[M+3H]^{3+}$ 739.3, Found: 739.5.



LCMS: 5-65%B over 20 min, rt = 9.56 min. HPLC: 5-65%B over 20 min, rt = 8.39 min. ESI-MS: Calcd. for $C_{98}H_{160}N_{24}O_{51}S$ $[M+2H]^{2+}$ 1261.5, Found: 1261.5, $[M+3H]^{3+}$ 841.4, Found: 841.5.



LCMS: 5-65%B over 20 min, rt = 11.40 min. HPLC: 5-65%B over 20 min, rt = 9.85 min. ESI-MS: Calcd. for $C_{73}H_{120}N_{22}O_{30}S$ $[M+2H]^{2+}$ 909.4, Found: 909.5, $[M+3H]^{3+}$ 606.6, Found: 606.8.



LCMS: 5-45%B over 20 min, rt = 10.36 min. HPLC: 5-45%B over 20 min, rt = 12.34 min. ESI-MS: Calcd. for $C_{116}H_{190}N_{24}O_{66}S$ $[M+2H]^{2+}$ 1504.6, Found: 1504.6. $[M+3H]^{3+}$ 1003.4, Found: 1003.7.

ABBREVIATIONS AND GLOSSARY

[0227] A: alanine

[0228] Ac: acetyl

[0229] ACT: α1-antichymotrypsin

Express Mail No.: EV 124826252 US
3602273v1
Attorney Docket No.: 2003080-0134
Client Reference No.: SK-1062-PRO3

[0230] Ala: alanine

[0231] Arg: arginine

[0232] Asn: asparagine

[0233] Asp: aspartic acid

[0234] Bn: benzyl

[0235] Boc: *tert*-butyloxycarbonyl

[0236] BPH: benign prostatic hyperplasia

[0237] BSP: benzenesulfinyl piperidine

[0238] Bu: butyl

[0239] Bz: benzoyl

[0240] CAN: ceric ammonium nitrate

[0241] coll: *sym*-collidine

[0242] C-terminus: peptide carbonyl terminus

[0243] Cys: cysteine

[0244] D: aspartic acid

[0245] DIEA: *N,N*-diisopropylethylamine

[0246] DMF: dimethyl formamide

[0247] DMSO: dimethyl sulfoxide

[0248] DTBMP: di-*tert*-butylmethylpyridine

[0249] DTBP: di-*tert*-butylpyridine

[0250] Et: ethyl

[0251] Fmoc: 9-fluorenylmethyloxycarbonyl

[0252] G: glycine

[0253] Gal: galactose

[0254] Glc: glucose

[0255] Gln: glutamine

[0256] Glu: glutamic acid

[0257] Gly: glycine

[0258] H: histidine

[0259] HATU: 7-azahydroxybenzotriazolyl tetramethyluronium hexafluorophosphate

[0260] His: histidine

[0261] Ile: isoleucine

[0262] K: lysine

[0263] kDa: kilodaltons

[0264] KLH: keyhole limpet hemocyanin

[0265] L: leucine

[0266] Leu: leucine

[0267] Lys: lysine

[0268] Man: mannose

[0269] MES-Na: 2-mercaptoethanesulfonic acid, sodium salt

[0270] MHC: major histocompatibility complex

[0271] N: asparagine

[0272] NAc: *N*-acetyl

[0273] NCL: native chemical ligation

[0274] N-terminus: peptide amine terminus

[0275] *O*-linked: linked through an ethereal oxygen

[0276] Pam3Cys: tripalmitoyl-S-glycerylcysteinylserine

[0277] PBS: phosphate-buffered saline

[0278] Ph: phenyl

[0279] Phth: phthalimido-

[0280] PMB: *p*-methoxybenzyl

[0281] Pro: proline

[0282] GP120: prostate specific antigen

[0283] Py: pyridine

[0284] QS21: a glycosteroidal immunoaduvant

[0285] R: arginine

[0286] S: serine

[0287] sat. aq.: saturated aqueous

[0288] Ser: serine

[0289] T: threonine

[0290] TBAF: : tetra-*n*-butylammonium fluoride

[0291] TBS: *tert*-butyldimethylsilyl

[0292] *t*Bu: *tert*-butyl

[0293] Tf: trifluoromethanesulfonate

[0294] THF: tetrahydrofuran

[0295] Thr: threonine

[0296] t-GP120: total prostate specific antigen

[0297] Trp: tryptophan

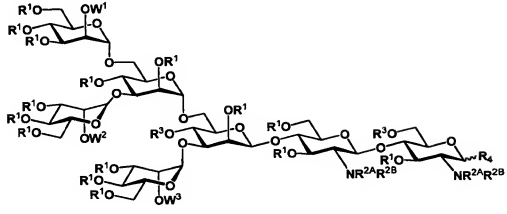
[0298] V: valine

[0299] Val: valine

[0300] W: tryptophan

CLAIMS

1. An isolated compound having the structure:

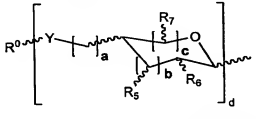


(I)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the

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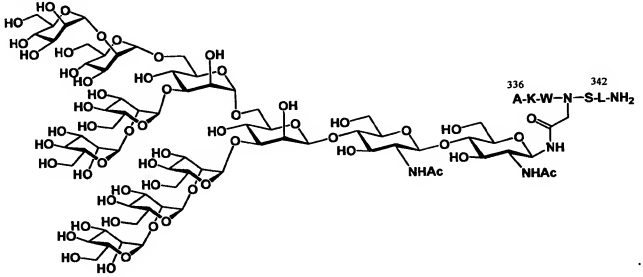
Client Reference No.: SK-1062-PRO3

nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

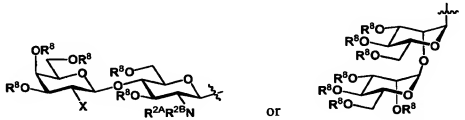
W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties;

and wherein R^4 is $-OR^{4A}$ or $-NHR^{4A}$; wherein R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N or O atom to which it is attached, either directly or through a crosslinker;

with the proviso that the compound is not a naturally occurring GP120 glycoprotein or a compound having the structure:

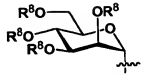


2. The compound of claim 1, weherein W^3 is R^1, R^3, as defined above, or a moiety having the structure:



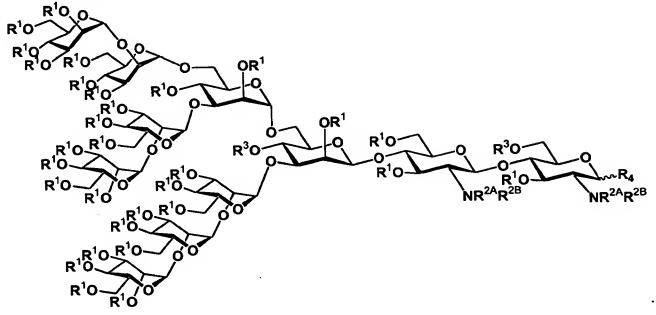
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

3. The compound of claim 1, weherein W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:

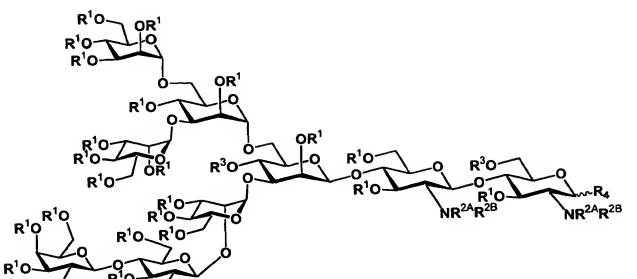


wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

4. The compound of claim 1 having the structure:



5. The compound of claim 1 having the structure:



;

wherein X is OR^1 or $NR^{2A}R^{2B}$.

6. The compound of claim 1, 4 or 5, wherein each occurrence of R^1 is independently an oxygen protecting group. In certain other exemplary embodiments, each occurrence of R^1 is independently hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, $-Si(R^{1A})_3$, $-C(=O)R^{1A}$, $-C(=S)R^{1A}$, $-C(=NR^{1A})R^{1B}$, $-SO_2R^{1A}$, wherein R^{1A} and R^{1B} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{1C}$ or $-ZR^{1C}$, wherein Z is –O-, -S-, $-NR^{1D}$, wherein each occurrence of R^{1C} and R^{1D} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

7. The compound of claim 6, wherein each occurrence of R^1 is independently hydrogen, alkylaryl, $-Si(R^{1A})_3$ or $-C(=O)R^{1A}$.

8. The compound of claim 7, wherein each occurrence of R^1 is independently hydrogen, Bn or Bz.

9. The compound of claim 1, 4 or 5, wherein for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently a nitrogen protecting group.

10. The compound of claim 1, 4 or 5, wherein each occurrence of $NR^{2A}R^{2B}$, R^{2A} and R^{2B} is independently hydrogen, alkyl, alkenyl, $-C(=O)R^{2C}$, $-C(=O)OR^{2C}$, $-SR^{2C}$, SO_2R^{2C}, or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; wherein each occurrence of R^{2C} is independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{2D}$ or $-ZR^{2D}$, wherein Z is –O-, -S-, $-NR^{2E}$, wherein each occurrence of R^{2D} and R^{2E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

11. The compound of claim 1, 4 or 5, wherein for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently $-C(=O)R^{2A}$ or SO_2R^{2A}; or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety.

12. The compound of claim 11, wherein for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently acyl, $-SO_2Ph$ or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted phthalimide moiety.

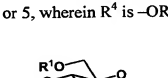
13. The compound of claim 5, wherein X is $-OR^1$:

14. The compound of claim 1, wherein each occurrence of R^3 is independently hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, -

$C(=O)R^{3B}$ or–ZR^{3B}, wherein Z is –O-, -S-, -NR^{3C}, wherein each occurrence of R^{3B} and R^{3C} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

15. The compound of claim 1, 4 or 5, wherein each occurrence R^1 and R^3 is independently hydrogen, alkylaryl, -$Si(R^{3A})_3$ or -$C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkylaryl, -$Si(R^{3A})_3$ or -$C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, -$C(=O)R^{3B}$ or–ZR^{3B}, wherein Z is –O-, -S-, -NR^{3C}, wherein each occurrence of R^{3B} and R^{3C} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

16. The compound of claim 1, 4 or 5, wherein R^4 is –OR^{4A} and the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; R^{4A} is hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, -$Si(R^{4B})_3$, -$C(=O)R^{4B}$, -$C(=S)R^{4B}$, -$C(=NR^{4B})R^{4C}$, -SO_2R^{4B}, wherein R^{4B} and R^{4C} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, -$C(=O)R^{4D}$ or–ZR^{4D}, wherein Z is –O-, -S-, -NR^{4E}, wherein each occurrence of R^{4D} and R^{4E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety; or R^{4A} comprises

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a protein, peptide or lipid moiety covalently linked to the O atom to which it is attached, either directly or through a crosslinker.

17. The compound of claim 1, 4 or 5, wherein R^4 is $-NHR^{4A}$ and the saccharide unit bearing R^4 has the structure:

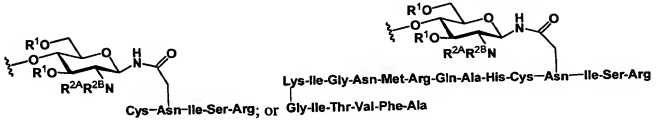
wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; and R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N atom to which it is attached, either directly or through a crosslinker.

18. The compound of claim 17, wherein R^{4A} is hydrogen.

19. The compound of claim 17, wherein R^{4A} comprises an amino acyl residue of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site.

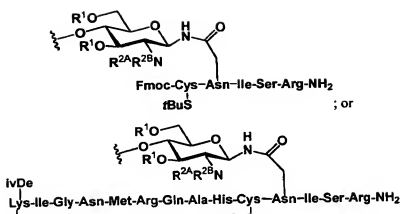
20. The compound of claim 19, wherein the amino acyl residue is Asn.

21. The compound of claim 1, 4 or 5, wherein R^4 is $-NHR^{4A}$ wherein R^{4A} comprises an Asparagine residue (Asn) of a peptide whose structure is either identical or closely related to that of GP120 near an N-glycosylation site and the saccharide unit bearing R^4 has the structure:

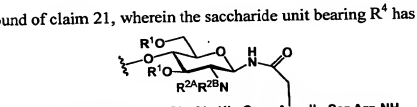


wherein any of the amino acid residues may bear one or more protecting groups.

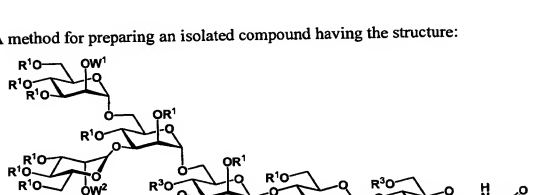
22. The compound of claim 21, wherein the saccharide unit bearing R^4 has the structure:



23. The compound of claim 21, wherein the saccharide unit bearing R^4 has the structure:



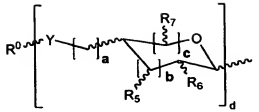
24. A method for preparing an isolated compound having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

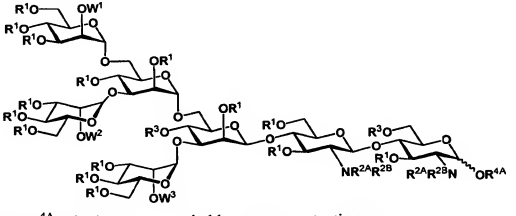


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties;

said method comprising steps of:

(a) providing an α-O-protected carbohydrate construct having the structure:



wherein R^{4A} is hydrogen or a suitable oxygen protecting group;

(b) reacting the construct of step (a) under suitable conditions to form a β-amino carbohydrate construct having the structure:

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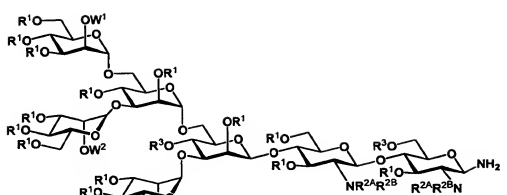
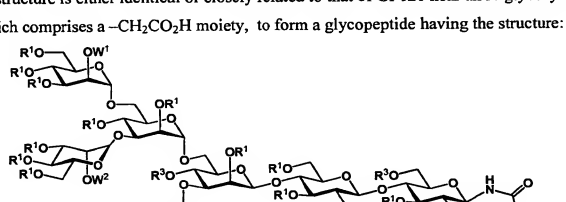
Attorney Docket No.: 2003080-0134
Client Reference No.: SK-1062-PRO3



(c) reacting said β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of GP120 near an N-glycosylation site and which comprises a $-CH_2CO_2H$ moiety, to form a glycopeptide having the structure:



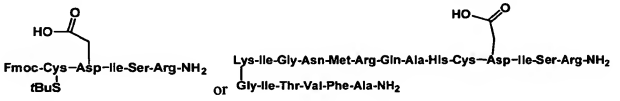
25. The method of claim 24, wherein in the step of reacting the carbohydrate construct of step (a) under suitable conditions to form the β-amino carbohydrate construct, Kochetkov amination conditions are used.

26. The method of claim 25, wherein NH_4HCO_3/H_2O is used.

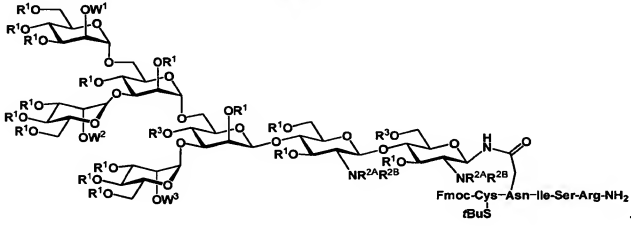
27. The method of claim 24, wherein, in the β-amino carbohydrate construct of step (b), each occurrence of R^1 and R^3 is hydrogen and each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

28. The method of claim 24, wherein, in the step of reacting the β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of GP120 near an N-glycosylation site, the reaction conditions comprise HATU and Hünig's base is a suitable solvent.

29. The method of claim 29, wherein the peptide has the following structure:

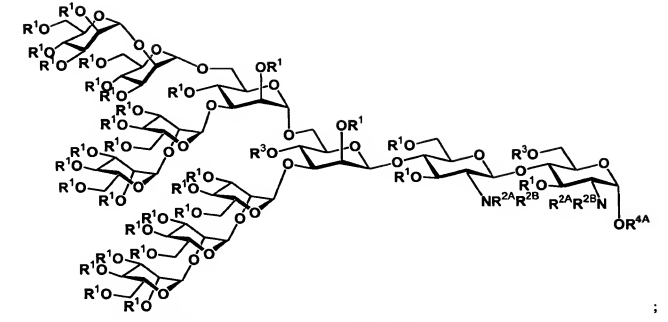


30. The method of claim 24, wherein the glycopeptide of step (c) has the structure:



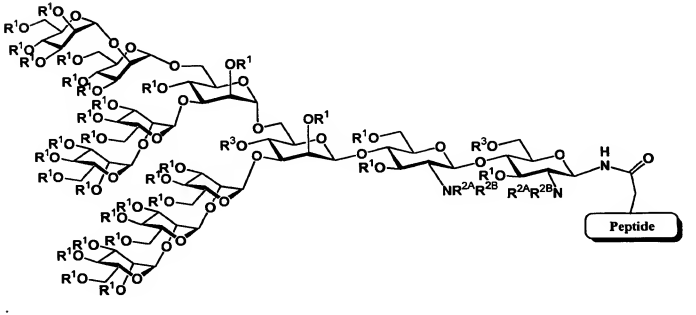
31. The method of claim 24, wherein, in the β-amino carbohydrate construct formed in step (b), each occurrence of R^1 is hydrogen, each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

32. The method of claim 24, wherein the α-O-protected carbohydrate construct of step (a) has the structure:



;

and the glycopeptide formed in step (c) has the structure:



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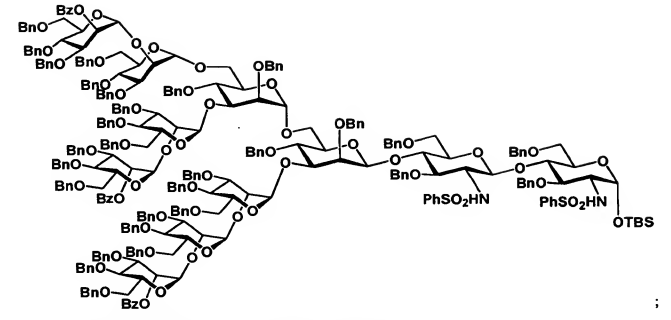
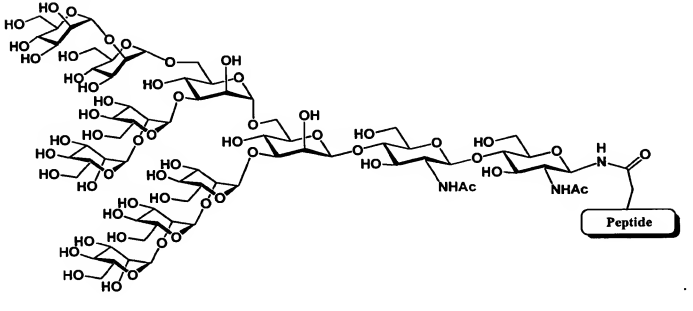
33. The method of claim 24, wherein the α-O-protected carbohydrate construct of step (a) has the structure:



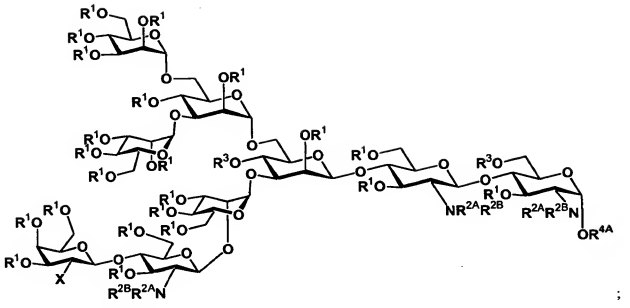
;

and the glycopeptide formed in step (c) has the structure:

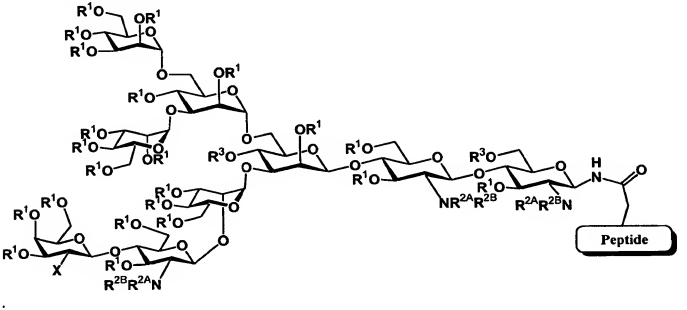


.

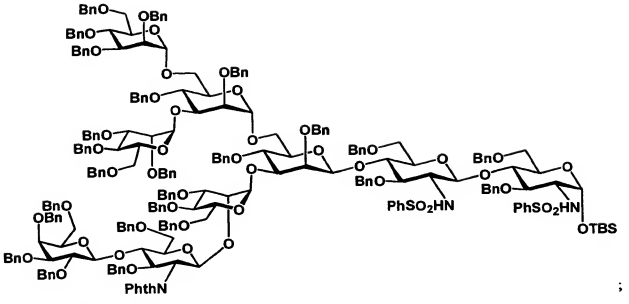
34. The method of claim 24, wherein the α-O-protected carbohydrate construct of step (a) has the structure:



and the glycopeptide formed in step (c) has the structure:

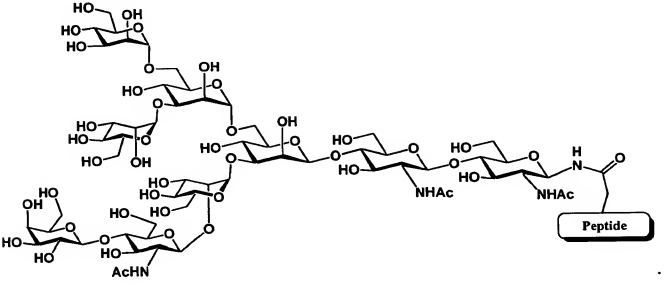


35. The method of claim 24, wherein the α-O-protected carbohydrate construct of step (a) has the structure:

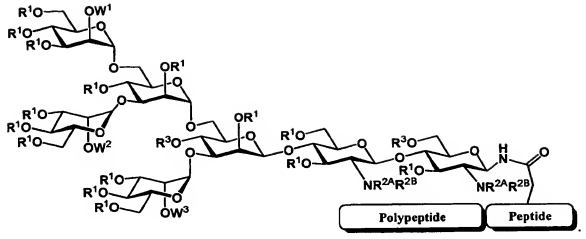


;

and the glycopeptide formed in step (c) has the structure:

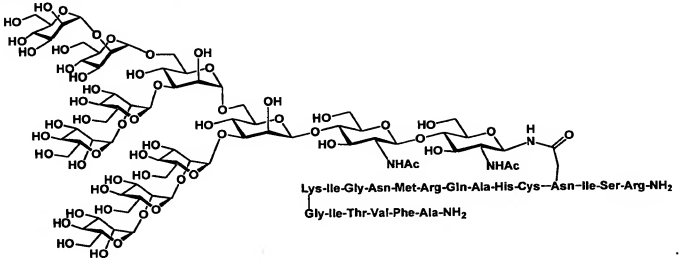


36. The method of claim 24, further comprising a step of subjecting the glycopeptide formed in step (c) to Native Chemical Ligation conditions in the presence of a suitable polypeptide to form a glycopolypeptide having the structure:



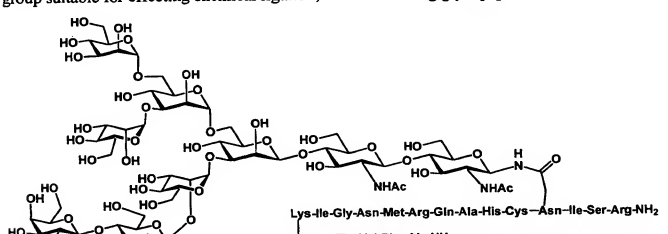
37. The method of claim 36, wherein the polypeptide comprises the amino acid sequence: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg wherein any one or more of the amino acid residues may bear one or more protecting groups or a moiety suitable for Native Chemical Ligation.

38. The method of claim 36, wherein the polypeptide has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His –SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:

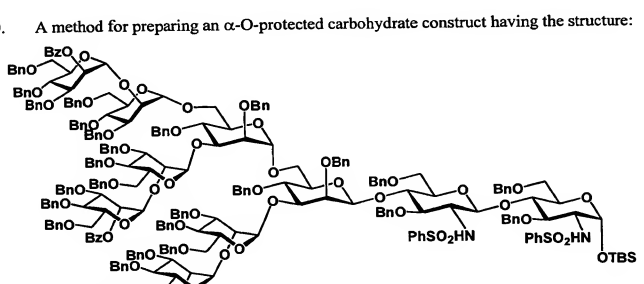


39. The method of claim 36, wherein the polypeptide has the structure:

Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His –SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



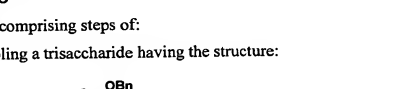
40. A method for preparing an α-O-protected carbohydrate construct having the structure:



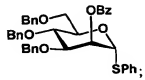
;

said method comprising steps of:

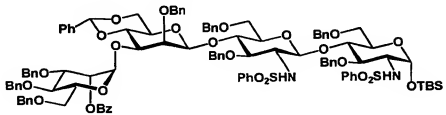
(a) coupling a trisaccharide having the structure:



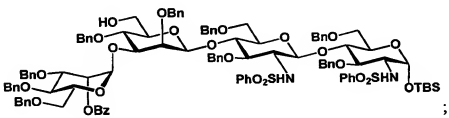
with a monosaccharide having the structure:



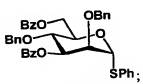
in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:



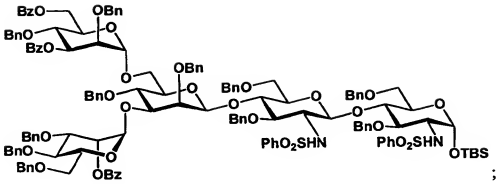
(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:



(c) coupling the partially deprotected tetrasaccharide formed in step (b) with a monosaccharide having the structure:

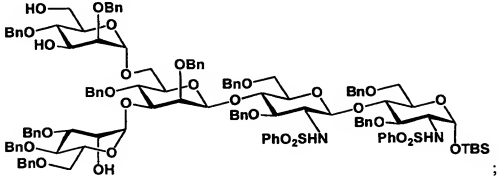


in the presence of an activating agent under suitable conditions to form a protected pentasaccharide having the structure:



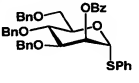
;

(d) partially deprotecting the pentasaccharide formed in step (c) under suitable conditions to form a partially deprotected pentasaccharide having the structure:

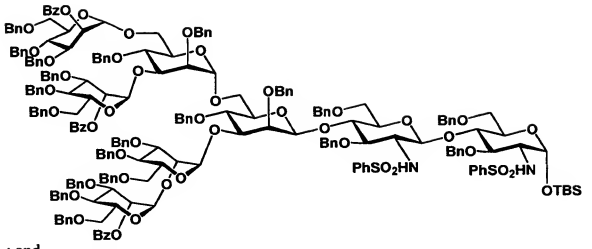


;

(e) coupling the partially deprotected pentasaccharide formed in step (d) with a monosaccharide having the structure:

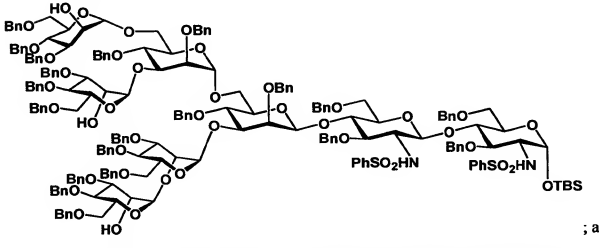


in the presence of an activating agent under suitable conditions to form an octasaccharide having the structure:



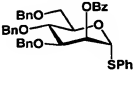
; and

(f) partially deprotecting the octasaccharide formed in step (e) under suitable conditions to form a partially deprotected octasaccharide having the structure:



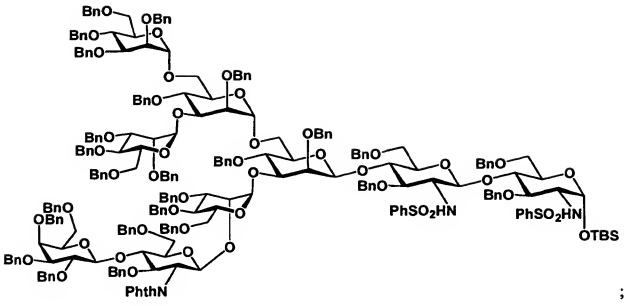
; and

(g) coupling the partially deprotected octasaccharide formed in step (f) with a monosaccharide having the structure:



in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

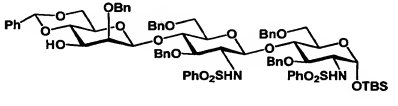
41. A method for preparing an α-O-protected carbohydrate construct having the structure:



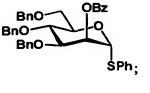
;

said method comprising steps of:

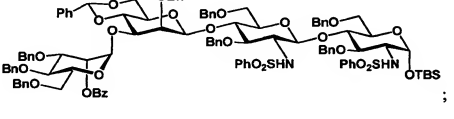
(a) coupling a trisaccharide having the structure:



with a monosaccharide having the structure:

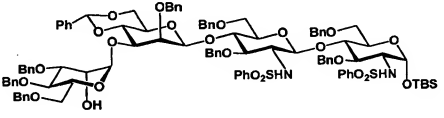


in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:

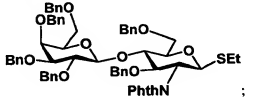


;

(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:

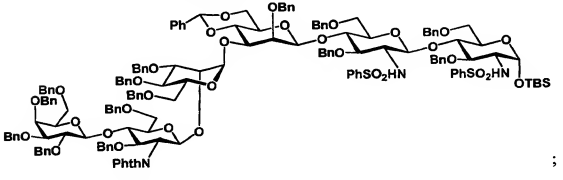


(c) coupling the partially deprotected tetrasaccharide formed in step (b) with an ethylthioglycoside having the structure:



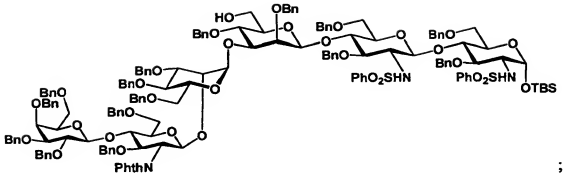
;

under suitable conditions to form a protected hexasaccharide having the structure:



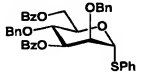
;

(d) partially deprotecting the hexasaccharide formed in step (c) under suitable conditions to form a partially deprotected hexasaccharide having the structure:

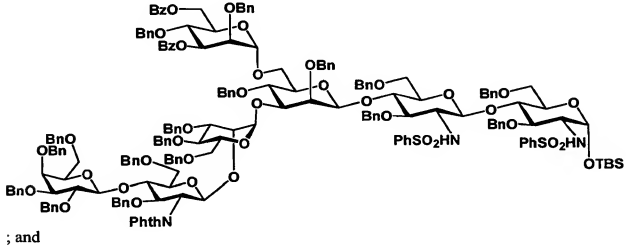


;

(e) coupling the partially deprotected hexasaccharide formed in step (d) with a monosaccharide having the structure:

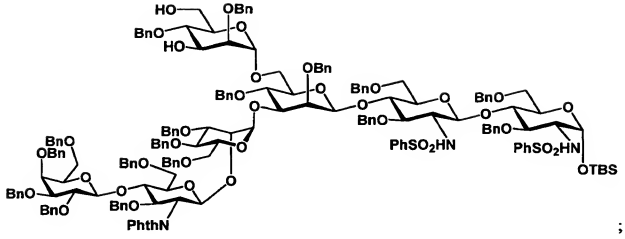


in the presence of an activating agent under suitable conditions to form an heptasaccharide having the structure:



; and

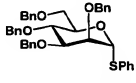
(f) partially deprotecting the heptasaccharide formed in step (e) under suitable conditions to form a partially deprotected heptasaccharide having the structure:



;

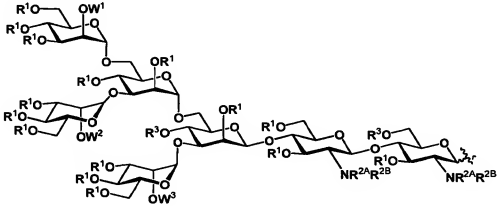
and

(g) coupling the partially deprotected heptasaccharide formed in step (f) with a monosaccharide having the structure:



in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

42. An antibody or antibody fragment which is specific to any one of the inventive antigens (independently of the others) containing a carbohydrate domain having the structure:

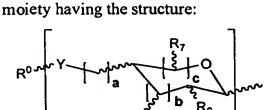


(I)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

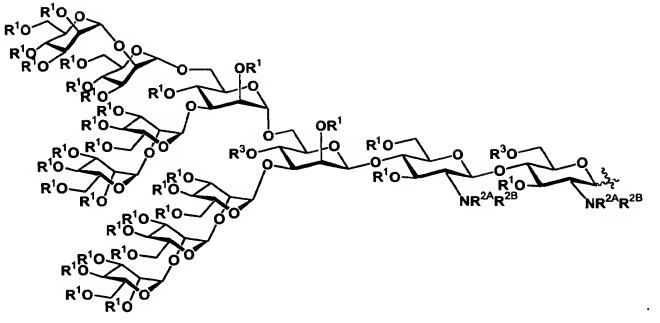


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

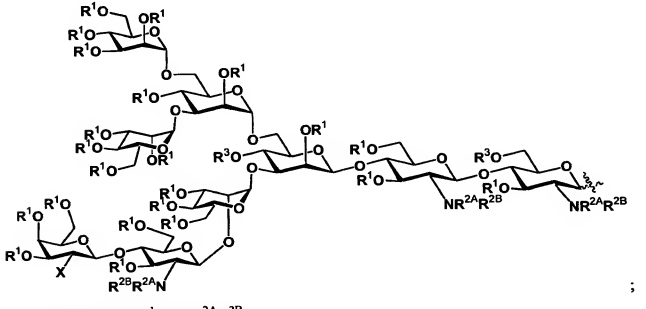
W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties;

and wherein said antibody is a purified polyclonal antibody or a monoclonal antibody.

43. The antibody or antibody fragment of claim 42, wherein, in the antigen, the carbohydrate domain has the structure:



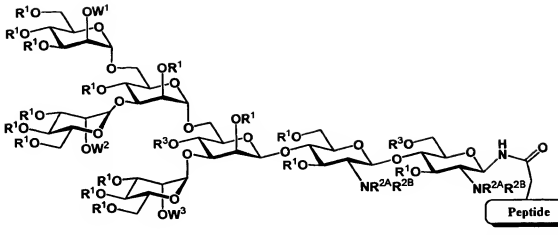
44. The antibody or antibody fragment of claim 42, wherein the antigen has the structure:



;

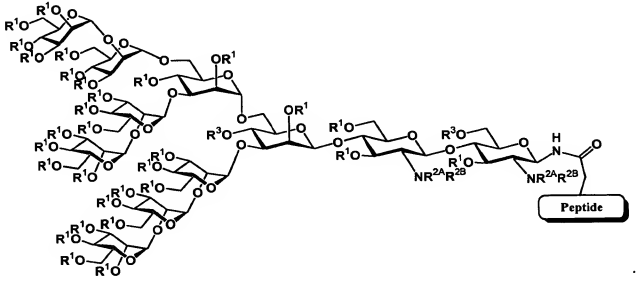
wherein X is OR^1 or $NR^{2A}R^{2B}$.

45. The antibody or antibody fragment of claim 42, wherein the antigen has the structure:

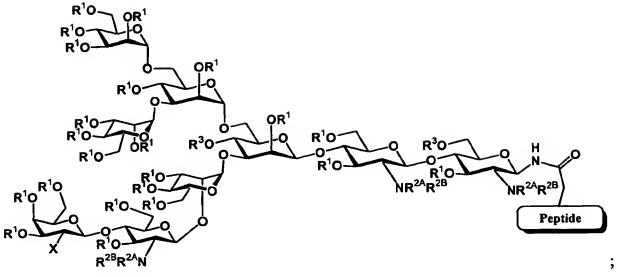


wherein the peptide has a structure either identical to or closely related to that of GP120 near an N-glycosylation site.

46. The antibody or antibody fragment of claim 45, wherein the antigen has the structure:



47. The antibody or antibody fragment of claim 45, wherein the antigen has the structure:

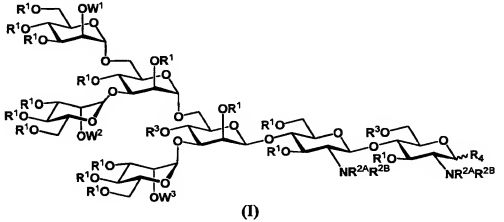


;

wherein X is OR^1 or $NR^{2A}R^{2B}$.

48. The antibody or antibody fragment of claim 42 or 45, wherein the antibody is a monoclonal antibody.

49. A pharmaceutical composition comprising an effective amount of a compound having the structure:

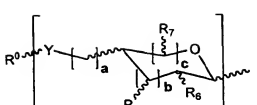


(I)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



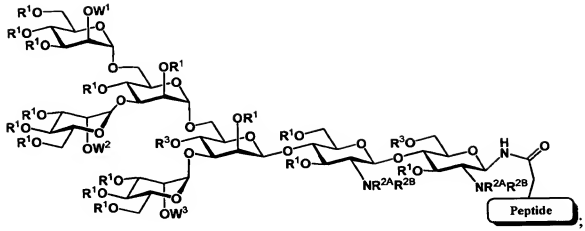
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties;

and wherein R^4 is $-OR^{4A}$ or $-NHR^{4A}$; wherein R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N or O atom to which it is attached, either directly or through a crosslinker;

in admixture with a pharmaceutically suitable diluent or carrier.

50. The composition of claim 49, wherein the compound has the structure:



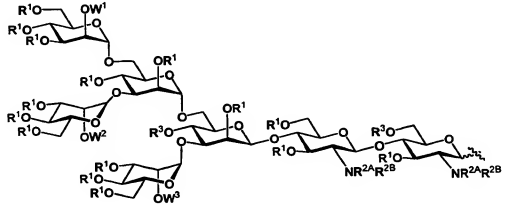
wherein the peptide has a structure either identical to or closely related to that of GP120 near an N-glycosylation site.

51. The composition of claim 50, wherein the N-glycosylation site is near Asn N295, N332, N339, N386 or N392.

52. The composition of claim 49 or 50, wherein the compound is covalently attached to a carrier immunostimulant.

53. The composition of claim 52, wherein the carrier immunostimulant is KLH, BSA or PamCys.

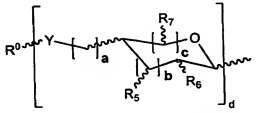
54. An antigenic construct comprising one or more carbohydrate domains having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

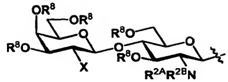
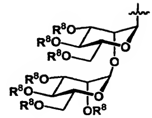


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose or galactose moieties

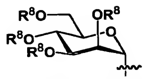
wherein each carbohydrate domain is independently covalently bound to a linker system, said linker system being a peptide or non-peptide nature.

55. The construct of claim 55, wherein W^3 is R^1, R^3, as defined in claim 68, or a moiety having the structure:

 or 

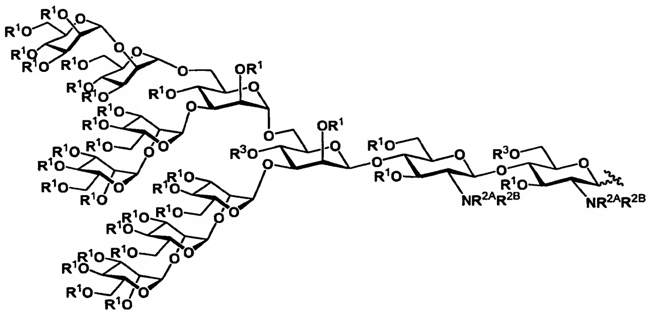
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

56. The construct of claim 54, wherein W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:

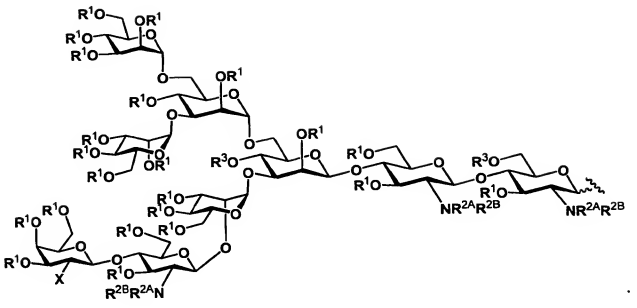


wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

57. The construct of claim 54, wherein one or more carbohydrate domains have the structure:



58. The construct of claim 54, wherein one or more carbohydrate domains have the structure:



59. The construct of claim 54, wherein the linker system is a peptide.

60. The construct of claim 54, wherein the linker system is designed to approximate the spatial position(s) of carbohydrate(s) in gp120.

61. The construct of claim 54, wherein the linker system is further attached to a carrier immunostimulant.

62. A composition comprising a construct of any one of claims 54-61, in admixture with a pharmaceutically suitable carrier.

63. The composition of claim 62, wherein the linker system is designed to approximate the spatial position(s) of carbohydrate(s) in gp120.

64. The composition of claim 62 or 63, wherein the linker system is further attached to a carrier immunostimulant.

65. A composition for eliciting an immune response in a subject comprising an effective amount of a compound of formula **(I), (II)** or **(III)** said amount being effective to induce antibodies in a subject;

in admixture with a suitable immunogenic stimulant.

66. The composition of claim 65 wherein the immunogenic stimulant comprises *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

67. A method of eliciting antibodies in a subject comprising administering to the subject a composition of any one of claims 54-61.

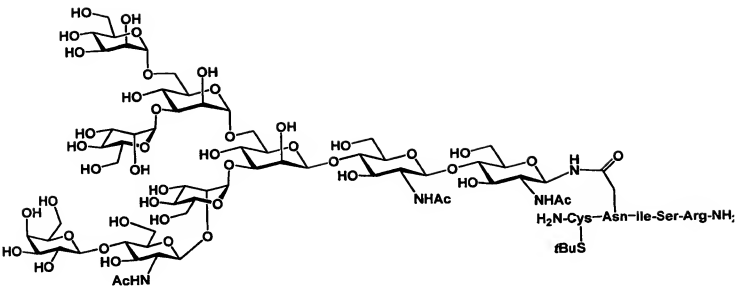
68. A method of preventing infection with HIV comprising administering to a subject an effective amount of a composition of any one of claims 54-61.

69. A method of treating an HIV infection comprising administering to a subject in need thereof an effective amount of a composition of any one of claims 54-61.

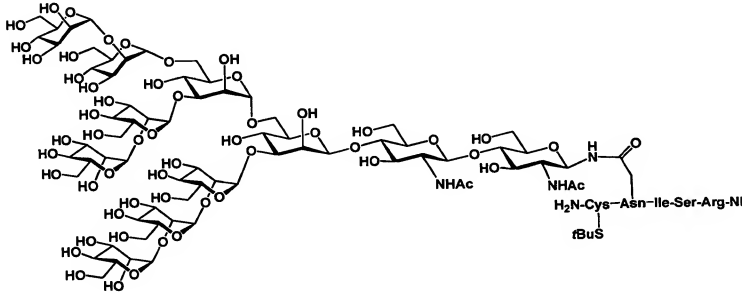
70. A method of eliciting an immune response wherein the response is directed against an antigen comprising a carbohydrate epitope expressed on the surface of gp120, said antigen having formula **(I), (II)** or **(III).**

71. The method of claim 70 further comprising administering an adjuvant.

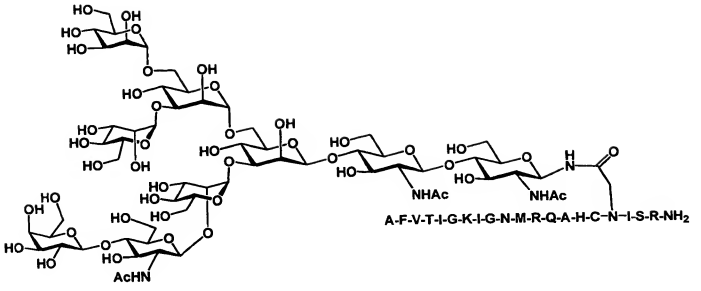
72. The compound of claim 1 having the structure:



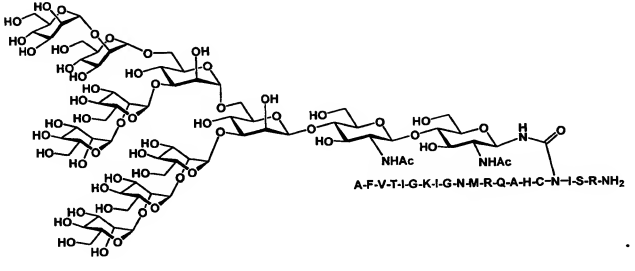
73. The compound of claim 1 having the structure:



74. The compound of claim 1 having the structure:

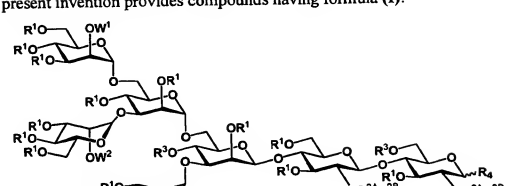


75. The compound of claim 1 having the structure:



ABSTRACT

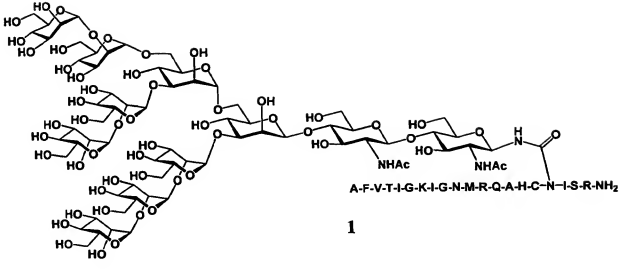
The present invention provides compounds having formula (I):



(I)

wherein R^1, R^{2A}, R^{2B}, R^3, R^4, W^1 , W^2 and W^3 are as defined herein; and additionally provides methods for the synthesis thereof, compositions thereof, and methods of use thereof in the treatment of HIV, methods for the prevention of HIV, and methods for inducing antibodies in a subject, comprising administering to a subject in need thereof, an effective amount of any of the inventive compounds as disclosed herein, either in conjugated form or unconjugated and in combination with a suitable immunogenic carrier. In another aspect, the invention provides an antibody or antibody fragment which binds specifically to a GP120 glycan or glycopeptide of the invention.

Figure 1



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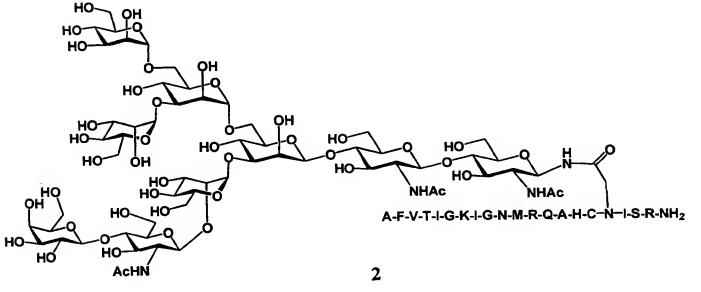

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